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The interaction of inflammation and coagulation systems

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ABSTRACT

Inflammation and coagulation are two systems playing pivotal roles in host defense. As phylogenetically old responses, there is extensive crosstalk between inflammation and coagulation in assuring adequate immune response against injurious stimuli. The crosstalk between activated coagulation and inflammatory mediators is highlighted. Inflammation activates coagulation and coagulation modulates the inflammatory activity in many ways. The contributing pathways are reviewed. Understanding the mechanisms involved in the crosstalk between inflammation and coagulation may yield new therapeutic strategies for human diseases. Immune cells are important in the initiation of coagulation pathways, while different inflammatory mediators could alter haemostasis. Vice versa, coagulation proteases have significant immuno-modulatory effects. The mechanisms controlling the interaction between inflammation and coagulation are described. Fibrinolysis is significantly involved in the inflammation-coagulation balance. Protease-activated receptors also play an important role in the

interaction between coagulation and inflammation. Elucidating the mechanisms of crosstalk between coagulation and inflammation increases our understanding of the pathological and pathophysiological events of severe clinical diseases, and may yield new therapeutic targets in the near future.

Keywords: coagulation, fibrinolysis, inflammation, innate immunity

1. INTRODUCTION

Inflammation is defined as "the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants that serves to: limit and control tissue damage, prevent infection by diluting toxins, initiate adaptive immune response and initiate healing by removing cellular debris" [1]. With the discovery of interleukins (IL), the concept of systemic inflammation developed. Despite the fact that the processes involved are identical to tissue inflammation, systemic inflammation is not confined to a given tissue, but involves the endothelium and other organ systems.

Coagulation (also known as clotting) is an ancient host defense system in invertebrates and vertebrates that limits the spread of pathogens [2, 3]. Phospholipid components called tissue factor (TF) and fibrinogen are released that initiate a chain reaction. A plug is immediately formed by platelets at the site of injury; this is called primary hemostasis; secondary hemostasis takes place concurrently: proteins in the blood plasma, named coagulation factors or clotting factors, respond in a complex cascade resulting to the formation of fibrin strands, which strengthen the platelet plug [4].

The coagulation cascade of secondary hemostasis has two pathways, the formerly known intrinsic and extrinsic pathways now defined as contact activation and tissue factor pathway, respectively, by which fibrin formation is achieved. In vertebrates, the coagulation system is composed of (a) coagulation proteases, such as thrombin, (b) activators, such as the transmembrane glycoprotein tissue factor, and (c) the final product, cross-linked fibrin [5, 6].

Tissue factor was initially defined as an initiator of blood coagulation, activating the coagulation cascade by formation of the ternary complex, a complex consisting of TF, the serine protease factor VIIa and zymogen factor X, which then is activated to factor Xa (FXa). The latter cleaves prothrombin into thrombin, which turns fibrinogen into fibrin monomers. During the past two decades TF has been recognized to have many other non-haemostatic roles in inflammation, tumor growth and angiogenesis [7]. The pivotal role of the TF/VIIa complex in pathophysiological processes, such as Gram-negative sepsis, neo-intimal hyperplasia after angioplasty, bacteremia, endotoxemia and coronary artery disease has been emphasized [8]. Expression of TF denotes a critical role in thrombosis and inflammation, exemplified with thrombotic complications in cancer, arteriosclerosis, septicaemia, and metastasis. A high risk of haemorrhage (a large flow of blood from a damaged blood vessel) or obstructive thrombosis (clotting) could be caused by coagulation disorders.

The activation of platelets and the coagulation cascade are kept under control through five mechanisms. Abnormalities can lead to an increased tendency toward thrombosis:

(1) Protein C also known as autoprothrombin IIA and blood coagulation factor XIV is a major physiological anticoagulant and a vitamin K-dependent serine protease enzyme. Thrombin binds to an endothelial cell membrane associated protein thrombomodulin (TM) and this complex converts protein C to its active form activated protein C (APC) ~1000 times faster than free thrombin [9]. The activated form, in collaboration with protein S and a phospholipid as cofactors, degrades the procoagulant factors of the prothrombinase and intrinsic Xase complexes, Va and VIIIa, respectively, thereby inhibiting coagulation [10]. Protein S appears to alter the cleavage site preferences of APC in factor Va, probably by changing the distance of the active site of APC relative to the membrane surface [11]. Constitutively expressed on endothelial cells, TM plays an important role in the intrinsic anticoagulant pathway being in parallel an anti-inflammatory agent [12]. APC also stimulates fibrinolysis by directly inactivating plasminogen activator inhibitor type 1 (PAI-1) [13]. Additionally, by limiting thrombin generation, APC suppresses in an indirect manner the thrombin-induced inactivation of PAI-1 [14] and the thrombin-mediated activation of a plasma procarboxypeptidase R (thrombin activatable fibrinolysis inhibitor, TAFI) [15]. APC also inhibits (a) the induction of TF on cells and (b) the thrombus formation that is a

result of the inflammatory insult. This is done by the suppressing of the inflammatory response [16].

(2) Antithrombin, a constantly active serine protease inhibitor (serpin), degrades the serine proteases (or serine endopeptidases): thrombin, FIXa, FXa, FXIa, and FXIIa. Quantitative deficiencies of antithrombin -or qualitative ones- lead to an abnormality of blood coagulation called thrombophilia [17].

(3) TFPI (Tissue Factor Pathway Inhibitor) inhibits factor Xa and factor VIIa bound to tissue factor. TFPI limits the action of tissue factor. Excessive TF-mediated activation of FIX and FX is also inhibited.

(4) The proteolytic cleavage of plasminogen (a plasma protein synthesized in the liver) generates plasmin. Tissue plasminogen activator (t-PA) which is synthesized and secreted by endothelium catalyzes the aforementioned cleavage. Fibrin degradation products inhibit excessive fibrin formation. These are generated by the proteolytic cleavage of fibrin by plasmin.

(5) Prostacyclin (prostaglandin I2 or PGI2) is a prostaglandin member of the eicosanoid family of lipid molecules and is also an effective vasodilator. It is released by endothelium and inhibits platelet Gs protein-linked receptors activation. This, in turn, activates adenylyl cyclase, leading to synthesis of cyclic adenosine monophosphate (cAMP). The way cAMP inhibits platelet activation is the decrease of cytosolic levels of calcium. By doing so, cAMP inhibits the release of granules that would cause the activation of additional platelets and the coagulation cascade [18].

For anticoagulation therapy, warfarin (and related coumarins) and heparin are the most usually used anticoagulants [19]. Warfarin, also known by the brand name Coumadin, has an effect on the vitamin K-dependent clotting factors II, VII, IX, X, whereas heparin and related compounds increase the antithrombin’s action on thrombin and factor Xa.

2. INFLAMMATORY RESPONSES AFFECT THE COAGULATION CASCADE

In humans, coagulation and inflammation ensure host survival by rising immediate responses to danger-associated signals derived from tissue injury, for example by

potentially harmful pathogens. The clotting system is activated either extrinsically by tissue damage outside the blood vessel or intrinsically by direct damage of vessels. Ca_2^{+-} or vitamin K-dependent factors afterwards operate in a protease-cascade manner leading to fibrin deposition and clotting. This response is modulated by a number of immunity-related mechanisms.

Cytokines are regulatory peptides with molecular weights <80 kD that mediate many different biological effects at picomolar concentrations. The cytokine network can be arbitrarily divided into three parts: proinflammatory cytokines, anti-inflammatory cytokines, and soluble inhibitors of proinflammatory cytokines. Tumor necrosis factor (TNF-α), interleukin (IL-1), IL-12, and interferon (IFN-γ), and other proinflammatory cytokines facilitate inflammation. The production of proinflammatory cytokines can be inhibited by anti-inflammatory cytokines, such as IL-4, IL-10, and IL-13, which can also exert a number of other inhibitory effects on inflammatory processes [20].

One further step in the modulation of the activity of proinflammatory cytokines can be made by naturally occurring inhibitors, such as soluble TNF receptors, soluble IL-1 receptors, and IL-1 receptor antagonist (IL-1ra). Innate immune receptors [Toll-like (TLRs) or NOD-like receptors (NLRs) both activate intracellular signaling cascades, resulting in the release of proinflammatory cytokines (e.g. IL-6, IL-1β, TNF-α) and recognize invading pathogens. Various proinflammatory cytokines can lead to decreased levels of anticoagulant proteins including thrombomodulin, the endothelial cell protein C receptor (EPCR), and protein S [21], resulting in an inflammatory, procoagulant state.

The coagulation cascade is modulated by cytokines via several mechanisms:

- Tissue factor and von-Willebrand factor (vWF) expression and release are increased. When the endothelium is damaged, the underlying protein von Willebrand factor is exposed to blood and recruits Factor VIII, collagen, and other clotting factors [22].

- Thrombomodulin is significantly downregulated by proinflammatory cytokines such as TNF-α and IL-1, resulting in diminished protein C activation [23]. TM switches the specificity of thrombin by mechanisms like conformational changes in thrombin, blocked access of normal substrates to thrombin and the provision of a binding site for protein C [24].

- The plasminogen activator inhibitor-1 (PAI-1) is upregulated, inhibiting lysis of fibrin clots [25].

- Neutrophils and platelets interact directly promoting clotting. Human immunodeficiency virus type I enhancer binding protein 1 (HIVEP1), a risk factor for venous thrombosis, may contribute to clotting by transcriptional regulation of inflammatory target genes [22].

The net impact of the proinflammatory response results in a pro-thrombotic shift of the clotting cascade. The net impact of inflammatory responses on the coagulation system results in (a) stimulation of coagulants, (b) decreased synthesis of anticoagulants, and (c) suppression of fibrinolysis.

3. THE CYCLE OF INFLAMMATION AND COAGULATION

Coagulation products generated directly by the inflammation accompanying the innate immune response (the role of which is to destroy an invading pathogen) perpetuate and enhance inflammatory reactions. Procoagulant activity is regulated by three anticoagulant pathways: antithrombin (AT), activated protein C (which is generated by thrombomodulin- thrombin complex on the endothelial cell), and tissue factor pathway inhibitor [26]. The function of all three pathways is probably to be damaged in the course of inflammation-induced activation of coagulation.

The inflammation-mediated increase in coagulation in conjunction to the ability of coagulation to amplify inflammation suggests that when appropriate controls for these two systems are lost, the mutual amplification of these systems contributes to the rapid onset of morbidity and mortality [27]. Excessive coagulation is controlled by natural anticoagulants: thrombomodulin and the protein C system, antithrombin and heparin-like proteoglycans on the vascular surface, tissue factor pathway inhibitor [10]. This is done by inhibiting factors Va and VIIIa, the serine proteases, factors IXa, Xa, and thrombin, and the tissue factor-factor VIIa complexes, respectively.

Inflammation activates coagulation by the following three mechanisms:

(1) cytokine induction of TF expression. Inflammation elicits coagulation primarily by activating the tissue factor pathway [28]. The responsible cytokine is mainly interleukin 6 (IL-6). Blocking IL-6 completely blocks enhanced thrombin formation

in endotoxemia [23]. Besides, TNF-α is a stimulus for TF expression [29] but its blockade does not affect endotoxemia-induced thrombin formation. IL-1 and IL-12 are also proinflammatory TF-inducing cytokines. Inflammation enhances TF availability by several ways. Loss of the integrity of endothelial cell-to-cell junctions during inflammation exposes the constitutively expressed TF in the adventitial layer of larger vessels [30]. The most important finding, however, is that the development of inflammation up-regulates the synthesis of TF in endothelial cells, in the secretory cells macrophages and monocytes, as well as in dendritic cells (a type of APC cells) [31]. Formation of micro-particles from endothelial, mononuclear, polymorphonuclear, and other cells exposed to blood is propagated by inflammation. Micro-particles rising from stimulated monocytes carry considerable TF amounts which can be picked up by other cells when micro-particles fuse to their cell surfaces [32]. Micro-particles per se are also pro-coagulant surfaces.

(2) protein C system is downregulated by inflammation. An inflammation-enhanced pro-coagulant effect arises from the interaction between platelets, leukocytes, and endothelium. During inflammation, platelets are activated via direct stimulation by endotoxin, by pro-inflammatory mediators including platelet activating factor (PAF) and by thrombin itself. Once activated, platelets, granulocytes and monocytes interact in a P-selectin-dependent manner to further stimulate monocyte TF expression [10, 33] and

(3) inhibition of fibrinolysis. Fibrinolysis has a significant involvement in the inflammation-coagulation balance. Fibrinolytic activators and inhibitors may affect inflammatory cell recruitment and migration, modulating the inflammatory response. Fibrinolytic activators tissue-type and urokinase plasminogen activators (tPA and uPA) are severely released during inflammation. In particular, uPA and uPAR (receptor of urokinase plasminogen) on leukocytes have an impact on the migratory potential of leukocytes, probably via the activation of extracellular matrix degrading enzymes elastase, plasmin, and metalloproteinases by uPA/uPAR. uPAR also exhibits protease-independent properties, involving transmembrane signal transduction after binding to various ligands, causing the production of cytokine and growth factor [34]. On the other hand, PAI secretion by endothelium is motivated by pro-inflammatory cytokines. This is a long-lasting response, resulting in the inhibition of fibrin removal within circulation. While defective, late fibrin removal may increase organ damage in sepsis,

the inflammation-induced inhibition of fibrinolysis is likely a rational part of the organism defence. Rapid functional enhancement of macrophage function by uPA/uPAR concurrently with the sealing of the insulted tissue by fibrin is probably beneficial. Fibrinogen and fibrin straightforwardly influence the production of proinflammatory cytokines and chemokines by mononuclear cells and endothelial cells [35].

Both the activation of coagulation and the deposition of fibrin caused by inflammation are considered that influence inflammatory activity at the site of injury or infection. Thus, the relationship is physiologically efficient. However, inflammation-induced coagulation may also contribute to a considerable degree to disease [16]. Since increased inflammation can amplify coagulation that, in turn, can enhance inflammation, the failure of natural anticoagulant mechanisms to adjust the clotting process would naturally increase the inflammatory process.

Important players in the interaction between coagulation and inflammation are protease-activated receptors (PARs), which are believed to play a key role in translating coagulation products into inflammatory signals [36]. Currently 4 types have been identified ((PAR-1 through PAR-4). PARs mediate various cellular reactions as cytokine release, expression of adhesion molecules, cell migration, or proliferation. Unlike other receptors, PARs are not activated by a soluble, external ligand. Proteases, such as activated coagulation factors, detach a defined part of the NH_2-terminal chain of the receptor, thereby inducing a conformational change of the receptor. The thrombin proinflammatory effects are exerted mainly through the PAR-1. Thrombin has high affinity for PAR-3 and PAR-4 as well. Thrombin thereby induces upregulation of various proinflammatory cytokines in vitro, including monocyte chemotactic protein-1, IL-6, IL-8, and macrophage migration inhibitory factor.

4. CONCLUSIONS

The interaction between coagulation and inflammation is a matter of intense research. The molecular links between inflammation and coagulation are unquestioned. Inflammation endorses coagulation by leading to intravascular tissue factor expression, deriving the expression of leukocyte adhesion molecules on the intravascular cell

surfaces, and down-regulating the fibrinolytic anticoagulant pathway and the protein C anticoagulant pathway. Thrombin, in turn, can promote inflammatory responses. This creates a cycle that progresses to vascular injury as occurs in septic shock. Most complex systems are regulated by product inhibition. This inflammation-coagulation cycle follows the same principle with regulatory mechanism being the protein C pathway. The molecular basis by which the protein C pathway functions as an anticoagulant is well established when compared to the mechanisms involved in regulating inflammation.

The processes of inflammation and coagulation are related in a complex way and may affect each other. This relation occurs at the levels of platelet activation, fibrin formation, and resolution as well as physiological anticoagulant pathways. Thorough understanding of the molecular mechanisms that play a role in the close relation between coagulation and inflammation may help identify new targets for therapies that can modify excessive activation of these systems.

A microvascular failure due to systemic inflammatory response to severe infection or sepsis and the activation of systemic coagulation may occur in combination. Nevertheless, this is not a one-way process where inflammation leads to coagulation, but both systems closely interact; thus, coagulation can also substantially modulate the inflammatory activity.

It has been observed that natural anticoagulant mechanisms have anti-inflammatory activity. Another observation is that the natural anticoagulant mechanisms are downregulated by inflammation. These twin notations may constitute an explanation why in the late stages of some diseases the progress is rapid. It can be suggested that natural anticoagulants might provide an effective treatment in acute inflammatory diseases such as sepsis. Obtaining a more detailed understanding of the impact of the natural anticoagulants on the progression of inflammation-initiated diseases will likely aid more rational application of natural anticoagulants in their treatment.

Nowadays, there is incomplete knowledge of both the signal transduction pathways and the biological functions transduced by the tissue factor and factor VIIa complex. So, it is necessary to investigate signal transduction pathways as well as biological functions mediated by TF/FVIIa, with special reference to gene induction, to the production of inflammatory markers and to cell migration.

Procoagulant material expressed in particular tissue factor by inflammatory cells may initiate activation of coagulation. The thrombin generated will both activate platelets and result in the formation of a platelet-fibrin thrombus.

On the basis of anticipated experimental data and future clinical studies outcomes, it could be expected that simultaneous modulation of coagulation and inflammation will be more successful than specific therapies aimed at one of these systems. More specifically, therapies aimed at tissue factor or at physiological regulatory pathways, such as the protein C system, may be most promising.

Revealing the molecular mechanism which regulates the expression of protease-activated receptors is important when new strategies referring to vascular diseases (prevention and treatment) are to be implemented.

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