Coagulation inhibitors in inflammation

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Abstract
Coagulation is triggered by inflammatory mediators in a number of ways. However, to prevent unwanted clot formation, several natural anticoagulant mechanisms exist, such as the antithrombin–heparin mechanism, the tissue factor pathway inhibitor mechanism and the protein C anticoagulant pathway. This review examines the ways in which these pathways are down-regulated by inflammation, thus limiting clot formation and decreasing the natural anti-inflammatory mechanisms that these pathways possess.

Introduction
Inflammatory mediators trigger the coagulation response in a number of ways: they induce tissue factor that initiates the coagulation cascade, they can activate cells causing the exposure of negatively charged phospholipids [1] on the outer membrane leaflet of the activated cell, and they can increase both platelet numbers and reactivity [2,3]. To prevent unwanted clot formation, there are several natural anticoagulant mechanisms that limit clot formation: the antithrombin–heparin mechanism, the tissue factor pathway inhibitor mechanism, and the protein C anticoagulant pathway. Evidence exists that inflammation can down-regulate these pathways and that these pathways themselves have multiple anti-inflammatory activities. Thus, when acute inflammation occurs, the inflammation not only triggers the formation of tissue factor, but also down-regulates both the capacity to limit clot formation and decreases the natural anti-inflammatory mechanisms.

A schematic representation of the coagulation cascade is shown in Figure 1. The activation of factors V, VIII and VII are not shown in order to simplify the presentation. When tissue factor comes in contact with blood, it can activate cells causing the exposure of negatively charged phospholipids [1] on the outer membrane leaflet of the activated cell, and they can increase both platelet numbers and reactivity [2,3]. To prevent unwanted clot formation, there are several natural anticoagulant mechanisms that limit clot formation: the antithrombin–heparin mechanism, the tissue factor pathway inhibitor mechanism, and the protein C anticoagulant pathway. Evidence exists that inflammation can down-regulate these pathways and that these pathways themselves have multiple anti-inflammatory activities. Thus, when acute inflammation occurs, the inflammation not only triggers the formation of tissue factor, but also down-regulates both the capacity to limit clot formation and decreases the natural anti-inflammatory mechanisms.

Key words: Blood coagulation, endothelial cell protein C receptor, inflammation, protein C, sepsis, thrombosis.

Abbreviations used: IL, interleukin; NF-κB, nuclear factor-κB; PAI-1, plasminogen activator inhibitor-1; PAR, protease activated receptor.

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Figure 1 | Model of the function of the protein C anticoagulant pathway

All of the reactions of the coagulation and protein C anticoagulation pathway occur at membrane surfaces, depicted below the factors. When the tissue factor is exposed to blood, factor VIIa binds and the complex activates either factor IX (IX) or factor X (X). The factor IXa–factor VIIa complex activates factor X (X) to factor Xa (Xa). The Xa then forms a complex with factor Va to convert prothrombin (pro) into thrombin (T). T then binds to thrombomodulin (TM) to form the protein C activation complex. Protein C (PC) binds to the endothelial cell protein C receptor (EPCR), if present, and this complex is activated by the T–TM complex. In the capillary, where there is no EPCR, the PC is activated directly by the T–TM complex. Activated protein C (APC) can remain bound to EPCR, but this complex does not seem to be capable of inactivating factor Va, presumably an indication that it is targeted to as yet unidentified alternative substrates. When APC dissociates from EPCR, it can then bind to protein S (PS). This complex inactivates factor Va or factor VIIIa, thus shutting down T formation and preventing blood clot extension. Factor VIIIa inactivation is augmented by factor V in a process that also requires PS. Factor V Leiden, a factor V mutation that impairs the function of the protein C pathway, cannot serve in this role. See the text for further discussion. Modified Figure reproduced from [54] with permission ©1995 The Federation of American Societies for Experimental Biology, U.S.A.

Inflammation decreases expression and function of natural anticoagulant mechanisms

The antithrombin–heparin pathway is especially sensitive to down-regulation by inflammatory responses. Transcription of thrombomodulin and the endothelial cell protein C receptor are inhibited by interleukin 1α, tumour necrosis factor α and endotoxin [15,16]. Down-regulation decreases protein C activation, thereby promoting thrombus formation. Neutrophil elastase cleaves thrombomodulin from the endothelial cell surface, generating a much less active form of thrombomodulin [17]. This decrease in protein C activation is partially due to the requirement for both thrombomodulin and the endothelial cell protein C receptor to be cellulary bound for the endothelial cell protein C receptor to increase protein C activation. Blocking protein C binding to the endothelial cell protein C receptor has been shown to decrease protein C activation nearly 20-fold in vivo [18]. In sepsis...
patients, both the endothelial cell protein C receptor and thrombomodulin can be severely down-regulated both as seen by decreased capacity of the patients to generate activated protein C [19] and by immunohistochemistry [20]. In addition, protein C levels decrease significantly in patients with severe sepsis. This is probably due to a combination of consumption and organ dysfunction. Clinically, there is a correlation between the degree of protein C reduction and the probability of a lethal event in sepsis patients [21].

Relatively little is known about the impact of inflammation on the tissue factor pathway inhibitor system, primarily because the vast majority of the inhibitor is bound to the blood vessel [22,23].

**Reduction in natural anticoagulant mechanisms increases the inflammatory response and cellular apoptosis contributing to thrombosis**

The antithrombin–heparin pathway can modulate cellular responses by altering cellular responses to stimuli, thereby decreasing the inflammatory response [24]. In cultured endothelial cells, antithrombin binding to these cells increases prostacyclin formation [25] and decreases NF-κB (nuclear factor κB) signalling [26]. Syndecan 4 is probably responsible for cellular signalling [26]. In addition, administration of antithrombin can prevent leukocyte recruitment in both sepsis and in ischaemia reperfusion models [27].

Thrombomodulin and activated protein C, two members of the protein C anticoagulant pathway, can reduce inflammatory responses. Thrombomodulin prevents thrombin from activating PARs (protease activated receptors) [28,29]. This is because the thrombomodulin and PAR binding sites on thrombin (anion binding exosite 1) overlap. Thus the presence of thrombomodulin on endothelium tends to decrease exposure of leukocyte adhesion molecules caused by thrombin activation of the PARs. When thrombin is bound to thrombomodulin it also gains the ability to activate a plasma procarboxypeptidase R (also known as thrombin activatable fibrinolysis inhibitor) [30]. This carboxypeptidase is an inhibitor of the complement anaphylatoxin, C5a [31,32] and bradykinin [32]. Inhibiting these vasoactive substances would be expected to diminish microvascular injury [33] and help to prevent sudden drop in blood pressure, two common features of sepsis.

Thrombomodulin has direct anti-inflammatory activities on the endothelium. The N-terminal lectin-like domain of thrombomodulin appears to dampen the mitogen-activated kinase and NF-κB responses in endothelium [34], suggesting that there is a receptor for this receptor.

Activated protein C can dampen NF-κB signalling in monocytes [35–37]. It also decreases the ability of inflammatory mediators to induce tissue factor formation in leukocytic cell lines [24,38,39]. This latter function is dependent on the endothelial cell protein C receptor. Activated protein C inhibits tight neutrophil adhesion to endothelium [40], probably due to a decrease in the formation of inflammatory cytokines. Activated protein C can also decrease endothelial cell apoptosis [41]. This function appears to involve both the endothelial cell protein C receptor and PAR 1 [42]. In mouse stroke models, activated protein C administration reduces brain damage, at least in part by inhibiting apoptosis through down-regulation of P53 [43]. Inhibition of P53 expression also appears dependent on the endothelial cell protein C receptor and PAR 1.

The structure of the endothelial cell protein C receptor suggests possible roles of the protein C pathway in regulating the immune response. The receptor shares considerable sequence identity to the MHC class I/CD1 family of molecules [16] (Figure 2). Structurally, similar to the CD1 family, the
endothelial cell protein C receptor has a tightly bound lipid, in this case a phospholipid, located in a region virtually identical with the antigen-presenting groove in the CD1 family [44]. Bacterially derived glycolipids bound to this groove in CD1 molecules can play a major role in the immune response to bacterial infection [45]. Of interest with respect to autoimmunity, deficiency of CD1d in mice leads to autoimmune disease [46]. Whether the endothelial cell protein C receptor plays a direct role in autoimmunity or the immune response to infection remains to be determined.

**Inhibition of fibrinolysis induced by inflammation**

Fibrinolysis is required for dissolving thrombi. Fibrinolysis occurs when plasminogen activation is initiated by plasminogen activators (tissue type and urokinase). Normally, these activators are inhibited by PAI-1 (plasminogen activator inhibitor-1). PAI-1 levels increase substantially in response to an inflammatory challenge [47]. The increase in PAI-1 levels results in more rapid inhibition of plasminogen activators and thus in severely impaired ability to remove the thrombus.

**Inflammation contributes to platelet numbers and responsiveness**

The inflammatory mediator, IL-6 (interleukin-6), increases platelet production and the newly formed platelets are more thrombogenic. For instance, they exhibit increased sensitivity to platelet agonists like thrombin [3]. Platelet activation contributes to the inflammatory response. Platelet activation releases concentrations of CD40 ligand. This protein then induces tissue factor synthesis [48,49] and increases levels of inflammatory cytokines such as IL-6 and -8 [50,51]. Thus, platelet activation can contribute to increased platelet number and responsiveness.

**Conclusions**

Under normal circumstances, the antiangiogenic mechanisms provide potent checks to prevent thrombosis. Inflammatory mediators promote coagulation by enhancing the initiation phase of coagulation, providing procoagulant membrane surfaces on which to amplify coagulation and by inhibiting natural antiangiogenic mechanisms. At the cellular level, inflammatory mediators can increase platelet numbers and reactivity. These interactions suggest a threshold phenomenon, as probably occurs in sepsis, when the procoagulant stimulus exceeds the capacity of the antiangiotants (now impaired) to control the process. Once this occurs, amplification of the coagulation system can proceed to the development of thrombosis and organ damage as a result. Poorly regulated coagulation probably amplifies the inflammatory process, causing additional organ injury.

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C.T.E. holds the Lloyd Noble Chair in Cardiovascular Research at OMRF (Oklahoma Medical Research Foundation).

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References


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