

The interactions between inflammation and coagulation

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Summary

Inflammation initiates clotting, decreases the activity of natural anticoagulant mechanisms and impairs the fibrinolytic system. Inflammatory cytokines are the major mediators involved in coagulation activation. The natural anticoagulants function to dampen elevation of cytokine levels. Furthermore, components of the natural anticoagulant cascades, like thrombomodulin, minimise endothelial cell dysfunction by rendering the cells less responsive to inflammatory mediators, facilitate the neutralisation of some inflammatory mediators and decrease loss of endothelial barrier function. Hence, downregulation of anticoagulant pathways not only promotes thrombosis but also amplifies the inflammatory process. When the inflammation–coagulation interactions overwhelm the natural defence systems, catastrophic events occur, such as manifested in severe sepsis or inflammatory bowel disease.

Keywords: protein C pathway, thrombosis, sepsis, inflammation, vascular biology

The blood clotting process

Under normal circumstances, the natural anticoagulant mechanisms provide a potent defence against thrombotic complications. Inflammatory mechanisms shift the haemostatic balance to favour the activation of coagulation and, in the extremes, either disseminated intravascular coagulation or thrombosis. Inflammatory mediators can elevate platelet count, platelet reactivity, downregulate natural anticoagulant mechanisms, initiate the coagulation system, facilitate propagation of the coagulant response and impair fibrinolysis. Similarly, clotting can increase the inflammatory response both by releasing mediators from platelets and by activating cells,

thereby promoting cell–cell interactions that increase the inflammatory responses.

A relatively traditional view of the blood coagulation cascade is depicted in Fig 1. In this cascade, the pathway is triggered when tissue factor, generally considered to be derived primarily from extravascular cells, comes in contact with factor VII/VIIa in the blood. The pathways are amplified by thrombin feedback activation of the cofactors factor V and factor VIII and the activation of the factor XI zymogen. As generally depicted, inflammation has little to do with the coagulation response.

The picture begins to change, however, in light of newer observations. *In vivo* (Palabrica *et al*, 1992; Falati *et al*, 2003) at wound sites and on artificial surfaces (Giesen *et al*, 1999; Rauch *et al*, 2000), blood-borne tissue factor seems to be responsible for the propagation of the thrombus. These studies implicate microparticles bearing tissue factor and P-selectin glycoprotein ligand-1 (PSGL-1, a leucocyte protein) as being essential for much of the build up of the thrombus at sites of injury. The concept is shown schematically in Fig 2. After injury, thrombin is generated at the wound site. Thrombin then recruits platelets and generates fibrin. The activated platelets expressing P-selectin bind to circulating microparticles released from leucocytes or vascular cells (Day *et al*, 2005). Inflammatory mediators presumably increase both the number of microparticles through leucocyte activation and the concentration of tissue factor on the particle surface. Since the microparticles are generated from the cell surface and are devoid of an ATP generating system, they will have high concentrations of negatively charged phospholipid on the membrane surface. In the absence of ATP, which is required for negative lipid flip-flop, there is no known way to remove this lipid from the outer membrane leaflet (Sims *et al*, 1988). As the particles flow over the developing thrombus, the negative membrane surface, rich in tissue factor, concentrates through PSGL-1-P-selectin interactions. Therefore, this leucocyte adhesion molecule that was originally believed to be mainly involved in leucocyte trafficking appears to play a dominant role in thrombus development.

In addition, artificial surfaces can lead to factor XII activation with subsequent activation of prekallikrein and the

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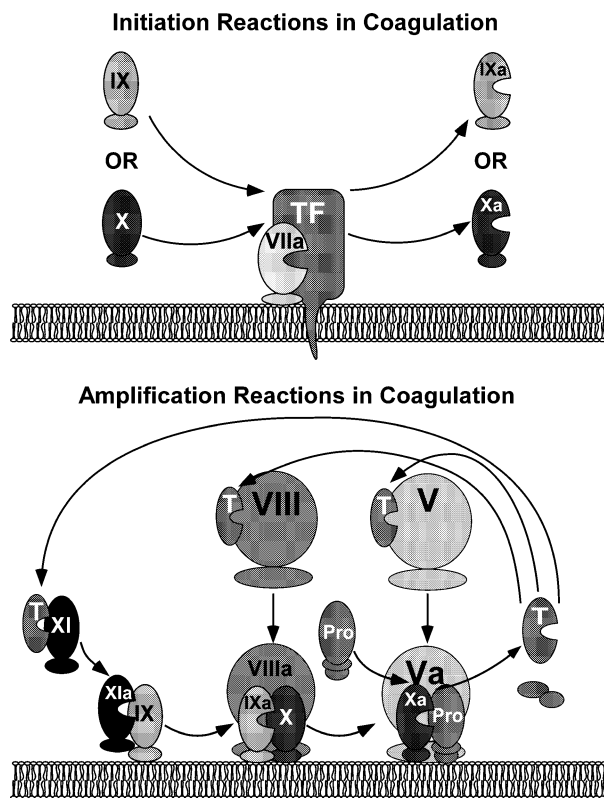


Fig 1. Initiation and amplification of blood coagulation. Top panel: Coagulation is initiated when tissue factor (TF) becomes exposed to blood. Tissue factor binds factor VII and the bound factor VII is very sensitive to activation by thrombin, factor Xa or neighbouring factor VIIa-tissue factor complexes (Neuenschwander *et al*, 1993). The tissue factor bound factor VIIa then converts either factor IX or factor X to their respective enzyme forms. Bottom panel: For sufficient thrombin to be generated for a clot to form, amplification is needed. This is accomplished on phospholipids and cellular surfaces expressing elevated levels of phosphatidylserine. This lipid is exposed only after exposure of cells to strong agonists. In addition to supporting tissue factor-factor VIIa mediated activities, these negatively charged phospholipids allow the amplification of coagulation reactions by a variety of mechanisms. First, factors IXa, VIIIa, X, Xa, prothrombin, and Va all bind independently to the surface. These binding interactions facilitate formation of the functional complexes between the enzymes, factors IXa and Xa, and the cofactors, factors VIIIa and Va. In the absence of the membrane surface, these direct protein-protein interactions are relatively weak. Furthermore, substrate binding (factors X, IX or prothrombin) results in increasing local concentration, positioning the substrate for optimal activation and perhaps conformational changes that also aid in activation. Not all events occur on the membrane surface. Thrombin, the terminal protease in the coagulation cascade, feeds back to activate factors V and VIII in the plasma, an essential event for these cofactors to be able to fully participate in the membrane associated activation events. Furthermore, thrombin activates factor XI, thereby providing an additional coagulation stimulus after the tissue factor-factor VIIa reaction has been inhibited by plasma inhibitors. Abbreviations: for simplicity, the factor name is not included before the roman numeral, TF, tissue factor; T, thrombin, Pro, prothrombin.

generation of bradykinin from high molecular weight kininogen. These events probably have little role in haemostasis, but

can contribute to vessel injury and vasodilation (Pixley *et al*, 1993) in a variety of diseases including severe sepsis.

Natural anticoagulant mechanisms

There are three major anticoagulant mechanisms that control the blood clotting process: tissue factor pathway inhibitor (TFPI), the heparin-antithrombin pathway and the protein C anticoagulant pathway. Deficiencies in the heparin-antithrombin or the protein C pathway in humans leads to increased risk of thrombosis, while the impact of deficiencies of the tissue factor pathway in humans is less clear (Dreyfus *et al*, 1991; Lane *et al*, 1996a; Lane *et al*, 1996b). The physiological significance of these pathways is demonstrated further by gene disruption experiments in mice. These result in embryonic or neonatal lethality when any single pathway is disrupted (Huang *et al*, 1997; Jalbert *et al*, 1998; Weiler-Guettler *et al*, 1998; Ishiguro *et al*, 2000; Gu *et al*, 2002).

TFPI inactivates factor VIIa bound to tissue factor. It utilises a unique mechanism. The inhibitor has two functional Kunitz domains. The second domain binds to and inhibits factor Xa, which because of its ability to bind negatively charged membranes, concentrates the TFPI-factor Xa complex on the membrane surface, at which time the first Kunitz domain of the inhibitor reversibly neutralises factor VIIa (Broze *et al*, 1988).

The antithrombin-heparin mechanism neutralises factor Xa, thrombin and factor IXa and factor VIIa bound to tissue factor (Rosenberg, 1987; Pabinger *et al*, 1992; Rezaie, 1998) (Fig 3). Antithrombin inhibition of the factor VIIa-tissue factor complex, factor IXa, factor Xa, and thrombin are all thought to be accelerated by vascular heparin-like proteoglycans (Marcum & Rosenberg, 1984).

The protein C pathway is triggered when thrombin binds to thrombomodulin on the endothelial cell surface, reviewed in (Esmon, 2003) (Fig 4). The thrombin-thrombomodulin complex activates protein C alone or more efficiently when protein C is bound to the endothelial cell protein C receptor (EPCR). Activated protein C (APC) retains binding affinity for EPCR. Once APC dissociates from EPCR it binds to protein S and proteolytically inactivates factors Va and VIIIa. Without these cofactors, factors Xa and factor IXa cannot effectively activate the downstream zymogen. Therefore, inactivation of the cofactors shuts off the coagulation system completely. In addition to generating APC, the thrombin-thrombomodulin complex accelerates the inactivation of thrombin by antithrombin and protein C inhibitor (Rezaie *et al*, 1995). Once the thrombin-inhibitor complex forms, conformational changes occur in thrombin (Baglin *et al*, 2002) that leads to its release from thrombomodulin, thus opening thrombomodulin to function.

The protein C pathway appears to be more complex than indicated above. Specifically, APC is a potent anticoagulant in reactions involving liposomes while it is a relatively poor anticoagulant on platelet surfaces (Solymoss *et al*, 1988). In model systems in which platelets are present over endothelium, it appears that APC anticoagulant functions better on the

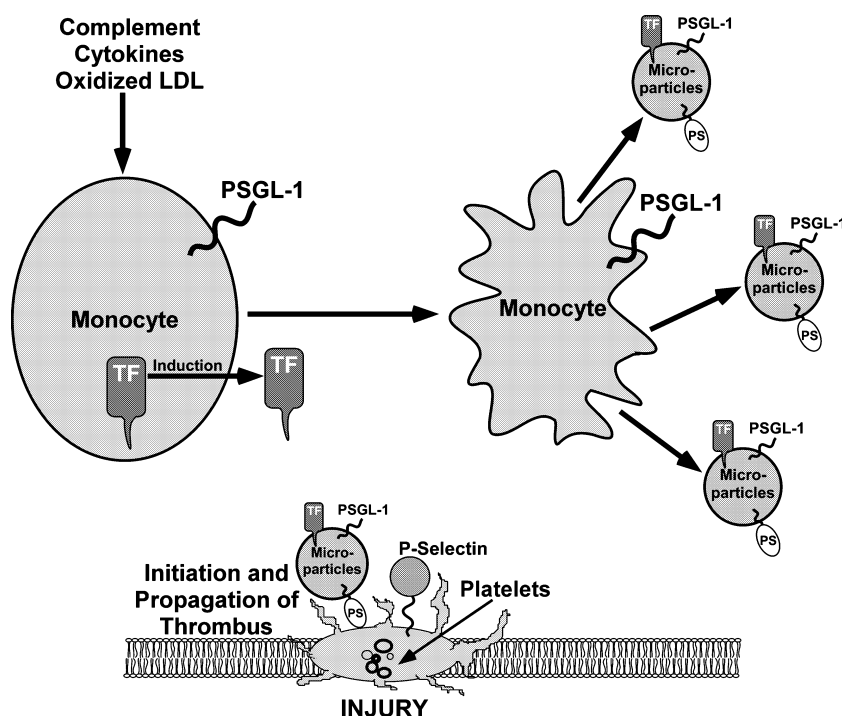


Fig 2. When monocytes are stimulated by cytokines or endotoxin, it triggers tissue factor (TF) synthesis, shape change and the release of micro-particles that contain the adhesion molecule, PSGL-1. The tissue factor-rich microparticles bind to activated platelets through the counter receptor P-selectin, thereby concentrating tissue factor at the wound site. The microparticles also have phosphatidylserine (PS) on the surface allowing the tissue factor to express potent coagulant activity and also allowing the factor IXa and Xa that is generated to amplify the coagulant response. Abbreviations: TF, tissue factor, LDL, low density lipoprotein.

endothelial cell rather than the platelet surfaces (Oliver *et al*, 2002). The components of the membrane surface responsible for these differences remain to be elucidated.

The impact of inflammation on coagulation

The multiple effects of inflammation on the haemostatic balance are summarised in Fig 5. One contribution of inflammation is to increase fibrinogen concentration. Fibrinogen, which is an acute phase reactant, is increased in inflammatory situations (Hantgan *et al*, 2001). Elevations in fibrinogen levels have been associated with an increase risk of thrombotic disease.

Probably the best known contribution of inflammation to coagulation is the induction of tissue factor expression on the cell surface of leucocytes, particularly monocytes (Parry & Mackman, 1998; Lindmark *et al*, 2000). Endotoxin, tumour necrosis factor- α (TNF- α) or CD40 ligand all induce tissue factor. If negatively charged phospholipids are exposed on activated monocytes, tissue factor gains major procoagulant activity. One approach to generating the negatively charged phospholipid surface is through microparticle formation, as described previously.

Inflammation also increases C reactive protein (CRP) concentrations in the blood. CRP has now been shown to facilitate monocyte-endothelial cell interactions (Han *et al*, 2004) and to promote plasminogen activator inhibitor-1 (PAI-1) and tissue factor (Cermak *et al*, 1993; Devaraj *et al*, 2003)

formation. Elevated CRP, probably a reflection of systemic inflammatory responses, is correlated with increased risk of myocardial infarction (Ballantyne *et al*, 2004). In experimental animals, elevated CRP increases injury from myocardial infarction (Danenberg *et al*, 2003; Paul *et al*, 2004).

Inflammation can contribute to platelet procoagulant activity. Exposure of the procoagulant lipids requires very potent agonists, such as thrombin plus collagen, calcium ionophores (Zwaal *et al*, 1989), or complement C5b-9 (Sims *et al*, 1989). CRP, an acute phase reactant, leads to complement activation (Wolbink *et al*, 1998) and thereby increases the available procoagulant membrane surface. Platelet activation by weaker agonists appears to result in limited expression of procoagulant lipid surfaces (Zwaal *et al*, 1989). Complement activation, apoptosis or necrosis brought on by inflammatory reactions can thus contribute to a hypercoagulable state by providing the key membrane surfaces on which the initiation and amplification aspects of coagulation can proceed.

Inflammatory mediators, such as interleukin (IL)-6, increase platelet production. The newly formed platelets appear to be more thrombogenic. For instance, the newly formed platelets activate at lower concentrations of thrombin (Burstain, 1997). Thus, both platelet count and platelet reactivity are increased in response to inflammatory mediators.

Platelet responsiveness can also be increased indirectly by inflammatory mediators. Histamine, TNF- α , IL-8 and IL-6 all lead to the release of ultra-large von Willebrand factor

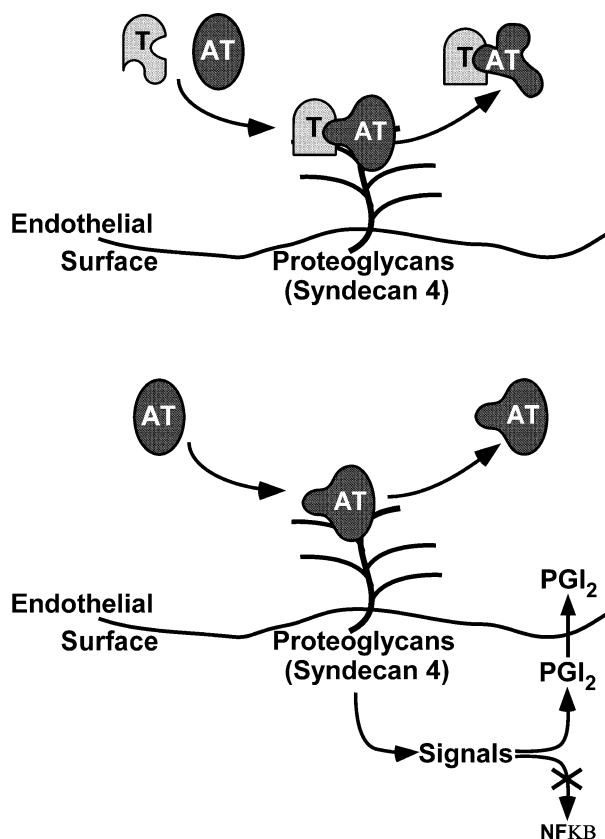


Fig 3. Antithrombin binds to heparin-like glycosaminoglycans, such as those on syndecan 4. Binding induces a conformational change in antithrombin (AT). Thrombin (T) and some other coagulation enzymes react preferentially with the bound antithrombin. In the case of thrombin this requires thrombin binding to the glycosaminoglycan (syndecan 4). Once thrombin binds to the inhibitor, major conformational changes occur that lead to the release of the complex (Baglin *et al*, 2002). In the absence of thrombin, antithrombin binding to the glycosaminoglycan leads to cell signalling, increasing prostacyclin (PGI₂) formation and decreasing NF-κB activation. See the text for a discussion.

multimers from the endothelium (Bernardo *et al*, 2004). These large multimers are particularly effective at promoting platelet thrombi at high shear rates. Normally, the high molecular weight multimers are processed by ADAMTS13 to generate less thrombogenic smaller forms. This process appears to be inhibited by IL-6 (Bernardo *et al*, 2004).

Inflammatory mediators can also induce expression of protease activated receptors (PARs) on endothelium (Nystedt *et al*, 1996). This in turn may make the cells sensitive to protease-mediated activation that could increase leucocyte adhesion molecules on the cell surface.

In acute inflammatory situations, such as sepsis, antithrombin is consumed and/or inactivated. Antithrombin inhibitory activity decreases markedly during severe sepsis, often to less than 50% of normal levels (Levi & ten Cate, 1999; Opal, 2000). Decreased antithrombin concentrations result in roughly proportional decreases in the rates of inhibition of the target

proteases. Thus, this decrease in antithrombin concentration results in delayed inhibition of coagulation enzymes that favour intravascular coagulation.

In addition to decreasing antithrombin concentration, the concentration of vascular heparin-like molecules can be reduced by inflammatory cytokines and neutrophil activation products (Klein *et al*, 1992). These *in vitro* findings have been extended to patient studies. In human severe sepsis, these heparin-like molecules have been shown to be downregulated or degraded (Klein *et al*, 1996), further diminishing the activity of this natural anticoagulant pathway.

Of the natural anticoagulant pathways, the protein C pathway appears to be especially sensitive to downregulation by inflammatory mediators. Endotoxin, IL-1 β , and TNF- α all downregulate both thrombomodulin and EPCR (Conway & Rosenberg, 1988; Fukudome & Esmon, 1994), thereby reducing the ability to generate APC (Fig 5). Neutrophil activation on the endothelial cell surface also downregulates the pathway. Neutrophil elastase cleaves thrombomodulin from the endothelial cell surface, generating a much less active form of thrombomodulin (Takano *et al*, 1990). In addition, protein C activation by soluble thrombomodulin is not enhanced by either cellular or soluble EPCR. Oxidants from the activated neutrophils can oxidise an especially sensitive methionine on thrombomodulin, greatly decreasing the activity of residual cellular thrombomodulin (Glaser *et al*, 1992). In septic patients, both the thrombomodulin and EPCR levels can be decreased severely as demonstrated immunohistochemically (Faust *et al*, 2001). Furthermore, the ability to activate protein C seems to be compromised in some septic patients (Liaw *et al*, 2003). In addition, protein C levels decrease dramatically in patients with severe sepsis. This is probably because of a combination of consumption and liver (the main site of protein C synthesis) dysfunction. Clinical observations indicate that the degree of protein C reduction correlates with a negative prognosis in septic patients (Fisher & Yan, 2000). Indeed, protein C supplementation has been found to decrease purpura fulminans (Rivard *et al*, 1995) in patients with meningococemia and apparently to increase survival (White *et al*, 2000a). The impact on the superficial thrombotic lesions is quite consistent with the demonstrated ability of protein C supplementation to reverse the purpura fulminans that develops in patients with severe protein C deficiency (Dreyfus *et al*, 1991).

Inflammation probably plays a role in vascular damage associated with bypass grafts and atherosclerosis. There is a rapid and very large downregulation of thrombomodulin when veins, but not arteries, are used for bypass grafts in rabbit models (Kim *et al*, 2002). The time course of thrombomodulin decrease correlates with increases in thrombosis and leucocyte infiltration (Kim *et al*, 2002). Support for the concept that thrombomodulin downregulation might be responsible for some of the thrombotic complications and leucocyte trafficking observed in the rabbit comes from studies by Waugh *et al* (1999, 2000). They found that over-expression of thrombomodulin on vessels that had been subjected to deep arterial injury reduced the throm-

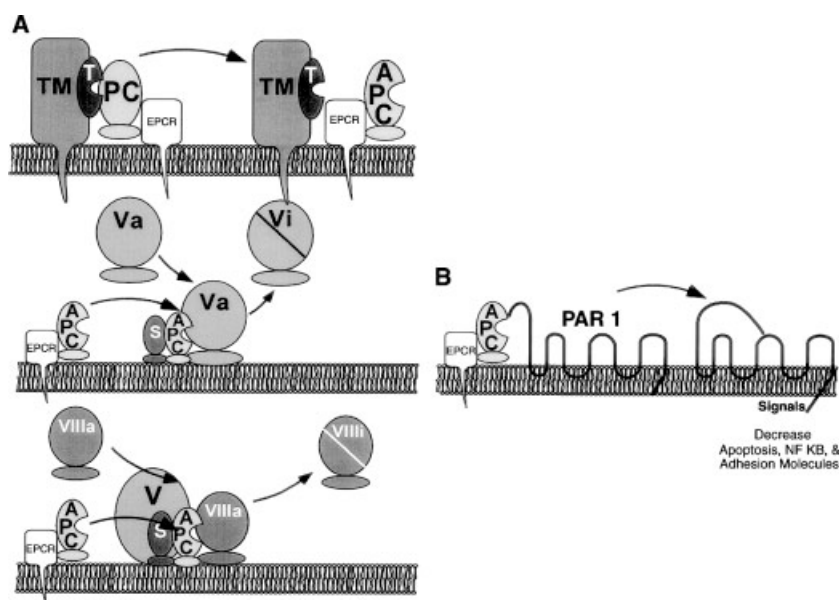


Fig 4. The protein C anticoagulant pathway. (A) Thrombin binds to thrombomodulin that in turn activates protein C. Protein C activation is augmented about 20-fold *in vivo* when it is bound to the endothelial cell protein C receptor (EPCR). Activated protein C (APC) and protein C bind to EPCR with comparable affinity. Once APC dissociates, it can bind to protein S to inactivate factors Va and VIIIa. Factor VIIIa inactivation is augmented by factor V. (B) The activated protein C-EPCR complex can cleave protease-activated receptor 1 (PAR 1), generating signals that inhibit apoptosis, decrease NF- κ B expression and decrease adhesion molecule expression (Joyce *et al*, 2001; Riewald *et al*, 2002).

biosis, leucocyte infiltration and subsequent neointimal formation. Conway *et al* (2002) have shown that the lectin-like domain of thrombomodulin dampens endothelial cell responses to inflammatory mediators and protects the endothelium from excessive leucocyte accumulation. Furthermore, this domain of thrombomodulin has been shown to bind and neutralise (Abeyama *et al*, 2005) high mobility group box 1 protein (HMGB1), a late stage mediator strongly contributing to organ failure in severe sepsis (Tracey, 2002).

Both thrombomodulin and EPCR expression (Laszik *et al*, 2001) are reduced on endothelium overlying atherosclerotic plaques. The mechanisms responsible for the downregulation are uncertain, but inflammatory cytokines generated within the lesion are good candidates. Of clinical interest, statins have been shown to limit TM downregulation, at least in cell culture (Esmon, 2000; Masamura *et al*, 2003). Furthermore, EPCR has been implicated in aiding APC to prevent loss of endothelial cell barrier function. This appears to be mediated, at least in part, by activation of sphingosine 1 phosphate receptor (Feistritzer & Riewald, 2005; Finigan *et al*, 2005). Increases in this receptor's function may be associated with APC's ability to activate protease-activated receptor 1 in an EPCR-dependent fashion (Feistritzer & Riewald, 2005).

The impact of coagulation on inflammation

Once tight control of thrombin and other coagulation enzymes is lost, they can participate in promoting the inflammatory

response (Fig 6). Platelet activation also appears to contribute to the inflammatory response. Platelets contain and are capable of releasing high concentrations of the pro-inflammatory mediator, CD40 ligand. This protein then induces tissue factor synthesis (Pendurthi *et al*, 1997; Miller *et al*, 1998) and increases inflammatory cytokines, such as IL-6 and IL-8 (Henn *et al*, 1998; André *et al*, 2002). Thus, inflammatory mediators leading to increased platelet number and responsiveness set in motion the ability to generate additional inflammatory responses.

Tissue factor-factor VIIa complex, especially when factor X is present, can activate PARs (Camerer *et al*, 1999; Isermann *et al*, 2001). This in turn can lead to the expression of adhesion molecules that facilitate leucocyte-mediated vessel injury. In addition, the tissue factor-factor VIIa complex can induce proinflammatory effects on macrophage/monocytes like the expression of major histocompatibility complex class II molecules and reactive oxygen species (Cunningham *et al*, 1999).

Fibrin, in addition to the proteases of the cascade, can participate in regulation of some aspects of inflammation. For instance, neutrophils bind tightly to fibrin when exposed to a gradient of TNF α or formyl-methionyl-leucyl-phenylalanine (FMLP) (Loike *et al*, 1995). This results in impaired leucocyte migration. In contrast, two other chemotactic agents, IL-8 and leucotriene B₄, fail to elicit tight leucocyte interaction with fibrin and the chemotactic response remains intact, suggesting a very specific response to fibrin elicited selectively by chemotactic agents.

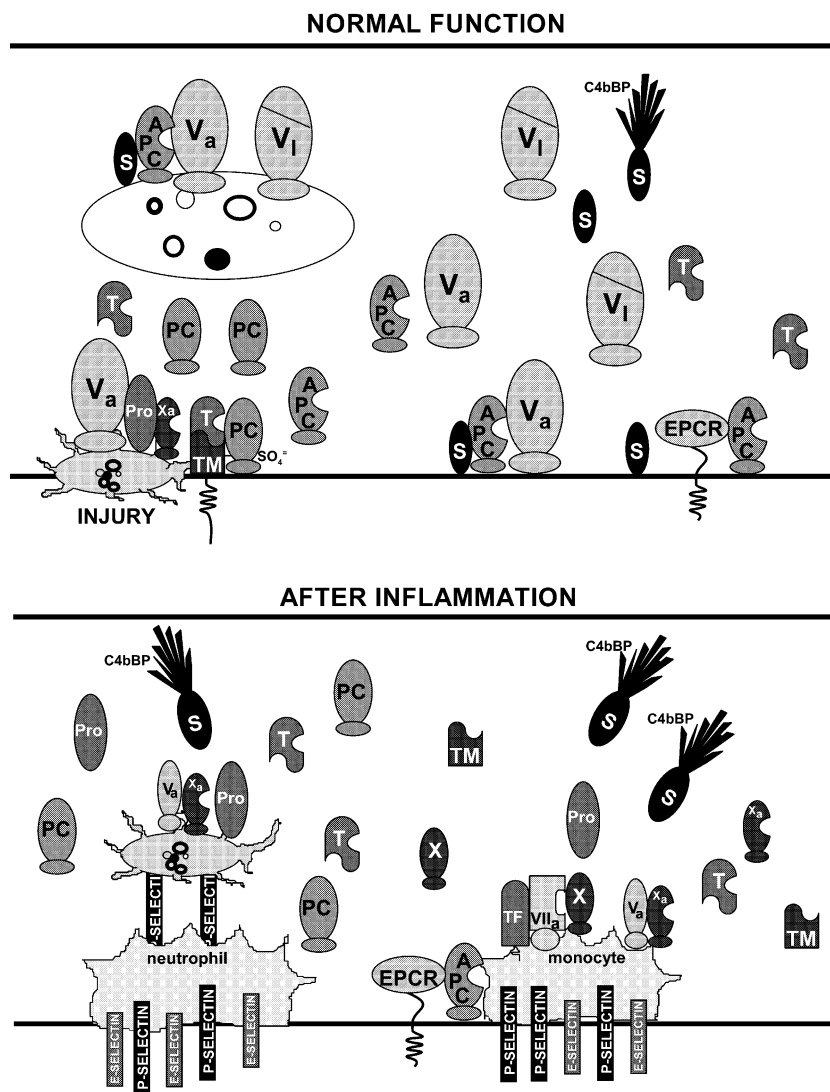


Fig 5. Differences between control of coagulation in normal *versus* inflamed vasculature. Top: The protein C anticoagulant pathway under normal conditions. Vascular injury initiates prothrombin (Pro) activation, which results in thrombin (T) formation. Prothrombin activation involves complex formation between factor Va (Va) and factor Xa (Xa). Thrombin then binds to thrombomodulin (TM) on the lumen of the endothelium, illustrated by the heavy line, and the thrombin-TM complex converts protein C (PC) to activated protein C (APC). The APC then binds to protein S (S) on cellular surfaces. The APC protein S complex then converts factor Va to an inactive complex (Vi), illustrated by the slash through the larger part of the two-subunit factor Va molecule. Protein C and APC interact with an endothelial cell protein C receptor (EPCR). Bottom: The protein C pathway after inflammation. In this model, inflammatory mediators lead to the disappearance of thrombomodulin from the endothelial cell surface. Endothelial cell leucocyte adhesion molecules, P-selectin or E-selectin, are synthesised or expressed on endothelial or platelet surfaces. Tissue factor (TF) is expressed on monocytes and binds factor VIIa (VIIa), and this complex converts factor X (X) to factor Xa (Xa), which forms complexes with factor Va (Va) to generate thrombin (T) from prothrombin (Pro). Because little APC is formed and the little that forms does not function well because of low protein S (S), factor Va is not inactivated and prothrombin activation complexes are stabilised.

The impact of natural anticoagulant mechanisms on inflammation and cellular apoptosis

In addition to inhibiting coagulant enzyme signalling through PARs, antithrombin can also modulate cellular receptor expression (Okajima, 2001). For instance, antithrombin downregulates the expression of CD11b/CD18 on leucocytes. Factor X binding to CD11b/CD18 augments factor X activation (Altieri *et al*, 1988; Altieri *et al*, 1991). Therefore, the

downregulation of this cellular factor X receptor would decrease both leucocyte adhesion and coagulation.

Antithrombin also inhibits cellular signalling. On cultured endothelial cells, antithrombin increases prostacyclin formation (Yamauchi *et al*, 1989) and decreases nuclear factor (NF κ B) signalling (Oelschläger *et al*, 2002). Antithrombin decreases both tissue factor and IL-6 expression in monocytes and endothelium (Souter *et al*, 2001). Anti-thrombin binding to syndecan 4, a proteoglycan on neutrophils, inhibits

CELLULAR AND HUMORAL RESPONSES TO nM CONCENTRATIONS OF THROMBIN

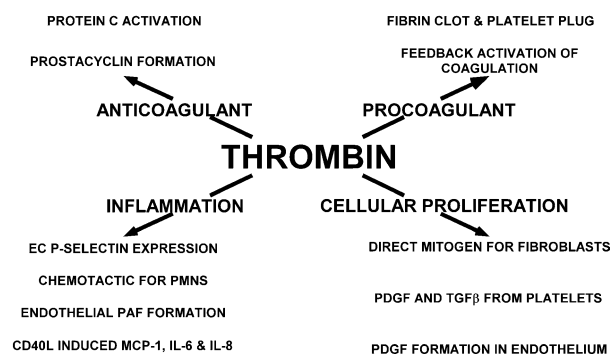


Fig 6. Thrombin is a multifunctional enzyme. Thrombin generates procoagulant, anticoagulant, inflammatory, and mitogenic responses. These responses serve to shift the haemostatic balance. Abbreviations: EC, endothelial cell; PMNs, polymorphonucleocytes; PAF, platelet activating factor; PDGF, platelet-derived growth factor; TGF- β , transforming growth factor- β ; CD40L, CD40 ligand; MCP-1, macrophage chemotactic protein-1; IL-6, interleukin 6; IL-8, interleukin 8.

chemokine-induced neutrophil migration (Kaneider *et al*, 2001). Interestingly, heparin blocks this effect. Consistent with these *in vitro* findings, administration of high levels of antithrombin to septic experimental animals and animals undergoing ischaemia reperfusion injury decreases leucocyte recruitment (Ostrovsky *et al*, 1997).

Several components of the protein C anticoagulant pathway reduce cellular inflammatory responses. Thrombomodulin prevents thrombin from activating PARs (Liu *et al*, 1991; Ye *et al*, 1992). Inhibition of PAR activation is achieved because the same site on thrombin (anion binding exosite 1) is responsible for PAR 1 and thrombomodulin binding.

The thrombin–thrombomodulin complex can also activate thrombin activatable fibrinolysis inhibitor (TAFI). This protein is sometimes referred to as procarboxypeptidase R. TAFI and protein C are roughly equivalent substrates for this activation complex (Bajzar *et al*, 1996; Wang *et al*, 2000). This enzyme removes C terminal Arg or Lys residues. The physiological significance of TAFI remains uncertain. Mice with the TAFI gene deleted are viable, appear physiologically normal and do not exhibit exaggerated thrombotic or inflammatory responses when subjected to a variety of stimuli (Nagashima *et al*, 2002). Originally, this enzyme was thought to function primarily by rendering fibrin resistant to clot lysis by removing C terminal Lys residues. Indeed, treatment of fibrin clots with the activated TAFI does make the clot somewhat more resistant to fibrinolysis. Recent studies have shown that by releasing C terminal arginine residues from target molecules, this carboxypeptidase serves as a very potent inhibitor of bradykinin (Myles *et al*, 2003) and the complement anaphylatoxin, C5a (Campbell *et al*, 2001; Myles *et al*, 2003). By inhibiting these vasoactive substances, the generation of this carboxypeptidase, especially in the microcirculation

(Esmon, 1989), probably helps to prevent severe drops in blood pressure, microvascular injury and oedema in severe acute inflammatory states. Potential roles in inflammation are suggested by the observation that TAFI levels appear to be downregulated by inflammatory mediators in humans whereas they are upregulated in mice (Boffa *et al*, 2003).

Thrombomodulin has recently been shown to have direct anti-inflammatory activity. Thrombomodulin is a multi-domain molecule that contains an N terminal lectin-like domain. This domain is not involved in protein C or carboxypeptidase R activation. However, when a mutant gene lacking the lectin domain is inserted in place of the normal thrombomodulin gene in mice and they are then challenged with endotoxin, the mutant mice recruit leucocytes much more aggressively than wild type mice (Pixley *et al*, 1993). Infusion of the isolated lectin domain can reverse this response (Pixley *et al*, 1993). Cell culture studies demonstrated that this domain dampened the mitogen-activated kinase and NF- κ B responses in endothelium (Pixley *et al*, 1993).

APC exhibits anti-inflammatory activity in part by inhibiting NF- κ B signalling in monocytes (Hancock *et al*, 1995; White *et al*, 2000b; Yuksel *et al*, 2002). APC also decreases inflammatory mediator-induced tissue factor expression on leucocytic cell lines (Shu *et al*, 2000; Murphy *et al*, 2001; Okajima, 2001) in an EPCR-dependent fashion. Tight neutrophil adhesion to endothelium that occurs in response to inflammatory mediators is also inhibited by APC (Sturn *et al*, 2003). Protein C and APC binding to EPCR also decreases leucocyte chemotaxis (Sturn *et al*, 2003).

The APC–EPCR complex can decrease endothelial cell apoptosis (Joyce *et al*, 2001), apparently through the activation of PAR 1 (Riewald *et al*, 2002). In mouse models of stroke, APC has been shown to minimise damage at least in part by inhibiting apoptosis through downregulation of P53 (Shibata *et al*, 2001). Inhibition of P53 expression was also dependent on EPCR and PAR1. Questions remain as to how activating PAR 1 generates anti-inflammatory activities. Indeed, deletion of the PAR 1 gene in mice has almost no apparent impact on sepsis, whereas inhibition of the protein C system increases death rate, cardiac failure, vascular collapse and elevates inflammatory cytokines (Esmon, 2005).

Fibrin and the plasminogen–plasmin system as mediators of inflammation

Fibrinogen and fibrin not only increase the production of pro-inflammatory cytokines and chemokines (including TNF- α , IL- β , and macrophage chemotactic protein-1) (Szaba & Smiley, 2002), but their levels are increased because of an acute phase response of fibrinogen. Increased fibrin formation occurs for the reasons described earlier. The tight linkage of this system with inflammation is clearly observed in fibrinogen-deficient mice. These mice show reduced macrophage adhesion and less cytokine production in models of inflammation (Szaba & Smiley, 2002).

Modulation of the fibrinolytic system by activators and inhibitors appears to have an effect on inflammatory cell recruitment and migration and the general inflammatory response. In particular, urokinase-type plasminogen activator (u-PA) and its receptor (u-PAR) are involved in these processes. u-PAR is involved in leucocyte adhesion to either the vessel wall or extracellular matrix. u-PAR binding to Mac-1 or vitronectin leads to increased cytokine and growth factor production (Blasi & Carmeliet, 2002). PAI-1 competes with u-PAR for binding to vitronectin, thus further decreasing cell adhesion and migration (Loskutoff *et al*, 1999). In u-PAR deficient mice, neutrophil recruitment in a bacterial pneumonia model is markedly reduced (Rijneveld *et al*, 2002).

The fibrinolysis inhibitors also play a role in modulating cytokine production. PAI-1 inhibits endotoxin-induced TNF- α production by mononuclear cells (Robson *et al*, 1990). TNF- α release from THP-1 cells in response to endotoxin is modulated by u-PA. Inhibition of u-PA decreases TNF- α release from THP-1 cells and increasing the u-PA concentration increases this response. Thus, like the coagulation system, the fibrinolytic system has multiple impacts on the inflammatory response.

Structural similarities between components of the coagulation/anticoagulation and inflammatory systems

The concept that coagulation can play a role in the inflammatory response is illustrated very well in the limulus crab (Iwanaga *et al*, 1998). In this system, endotoxin in the blood leads to activation of the coagulation system that, in turn, plays a direct role in walling off and controlling the infection.

The shared nature of the coagulation and inflammation systems is reflected in the structural motifs and proteins that are shared between the two systems. One example is the interaction of human protein S with C4 binding protein (C4BP), a regulatory protein of the complement system (Dahlbäck *et al*, 1990). When protein S is bound to C4BP, the ability of protein S to enhance APC anticoagulant activity is lost (Dahlbäck, 1986). Presumably, this complex interacts with negatively charged phospholipid membrane surfaces on cells to minimise attack by complement components. Surprisingly, although C4BP can contribute to the regulation of human coagulation, the protein S-C4BP complex is absent from rabbits (He & Dahlbäck, 1994) and cattle (Dahlbäck, 1986), suggesting a late evolutionary change that presumably offered some advantage to humans, probably with respect to some infectious disease.

EPCR shares striking structural similarities with the major histocompatibility class I/CD1 family of molecules both at the primary sequence (Fukudome & Esmon, 1994) and three-dimensional structural level (Oganesyan *et al*, 2002). The crystal structure of EPCR reveals it has a tightly bound phospholipid located in a region that corresponds to the antigen presenting groove (Fig 7). In the CD1 proteins,

glycolipids bind in this groove and can participate in the immune response to bacterial infections, such as tuberculosis (Moody *et al*, 2000). Deficiency of CD1 family members in mice leads to autoimmune disease (Hong *et al*, 1999). The structural similarities between EPCR and the CD1 family members suggest that it may also play a role in auto immunity and in host defence against infection. Blocking EPCR–protein C interaction, for instance, increases the animals' inflammatory and coagulopathic response to *E. coli* infusion. In addition, autoantibodies to EPCR are relatively frequent and correlate with autoimmune mediated fetal loss (Hurtado *et al*, 2004), consistent with a role for EPCR in immune regulation.

In thrombomodulin, the N terminal lectin-like domain (Conway *et al*, 1997) has homology with other lectin containing proteins, in particular the selectins. Whether this domain



Fig 7. The recombinant soluble endothelial cell protein C receptor (rsEPCR) molecule with a portion of the protein C Gla domain and a lipid molecule. In EPCR (yellow ribbon), two alpha-helices and an eight-stranded beta-sheet create a groove that is filled with phospholipid (the space filling balls in the centre). Binding of Ca²⁺ ions (magenta spheres) to the protein C Gla domain (green ribbon) exposes the N-terminal 'omega' loop, which, in the absence of EPCR, interacts with the phospholipid surfaces on the membrane. There do not appear to be direct interactions between the protein C Gla domain and the lipid molecule located in the groove of rsEPCR. The C and N terminal residue locations are indicated on the figure. The C terminal of the truncated EPCR points toward the membrane surface and is connected by a short region to the transmembrane domain of EPCR. From Oganesyan *et al* (2002). Reproduced with permission© 2002 The American Society of Biochemistry and Molecular Biology.

plays a role in carbohydrate recognition that is important for thrombomodulin cellular trafficking (Conway *et al*, 1997), protective functions related to endothelial cell cytokine generation (Conway *et al*, 2002) or cell adhesion (Huang *et al*, 2003) seems likely, but remains to be proven.

Tissue factor also has homology with components of the inflammatory system. In this case, tissue factor has structural similarities with the cytokine receptors (Edgington *et al*, 1991).

Finally, Mac-1, also known as CD11b/CD18, functions in a dual role on neutrophils and monocytes. It is an integrin involved in forming tight cell–cell binding interactions. It also promotes factor X activation by tissue factor–factor VIIa (Altieri & Edgington, 1988; Altieri *et al*, 1991). This integrin also binds proteinase 3, the auto-antigen in Wegener's granulomatosis, and soluble EPCR (Kurosawa *et al*, 2000). Presumably binding of EPCR to Mac-1 inhibits leucocyte interactions with activated endothelium.

The structural similarities between components of the inflammatory response systems and the coagulation system suggest parallel evolution and may explain some of the functional interactions described above.

The vicious cycle of inflammation and coagulation

Since increased inflammation can increase coagulation that, in turn, can enhance inflammation, the failure of natural anticoagulant mechanisms to control the clotting process would naturally increase the inflammatory process. The twin observations that inflammation downregulates the natural anticoagulant mechanisms and that these mechanisms have anti-inflammatory activity above and beyond their anti-thrombotic functions further exacerbates the situation. This suggests that in acute inflammatory diseases, such as sepsis, natural anticoagulants might provide an effective treatment. Indeed, antithrombin (Emerson *et al*, 1989), APC (Taylor *et al*, 1987) and TFPI (Creasey *et al*, 1993) have all been effective in preventing death from lethal levels of *E. coli* in a baboon model of sepsis. All of these were also tested in large clinical trials of patients with severe sepsis. Of these, only APC was effective in reducing 28 d all cause mortality (Bernard *et al*, 2001). Of interest, antithrombin (Warren *et al*, 2001) and TFPI both failed large randomised clinical trials (Abraham *et al*, 2003). The basis for the different results in trials with natural anticoagulants might lie in the dose/duration of treatment chosen, some of the differences in activities beyond their anticoagulant function as discussed above, or differences in responses in patients with sepsis and other co-morbidities, such as hypertension, diabetes or atherosclerosis. The impact of co-morbidities on sepsis outcomes in animal studies is almost never analysed. Other potential differences in the animal and patient studies have been discussed recently (Esmon, 2004). A more detailed understanding of the impact of the natural anticoagulants on the progression of inflammation initiated diseases will

aid substantially in understanding when and how to use natural anticoagulants as therapeutics.

Clinical conditions where inflammation and coagulation combine to influence disease progression or outcome

The inflammation/coagulation interface discussed above probably participates in a variety of disease processes. This is most clear in severe sepsis, but emerging data also suggests an important role in inflammatory bowel disease (Faioni *et al*, 2004). Patients with rheumatoid arthritis exhibit increase risk of myocardial infarction (Maradit-Kremers *et al*, 2005), potentially providing another mechanism in which inflammation contributes to thrombosis—specifically athero-thrombosis. Furthermore, the general concept of inflammation-mediated downregulation of natural anticoagulants can be seen in common diseases, such as atherosclerosis and diabetes. Several features, such as elevation of CRP and IL-6, are common to most of these disease processes (Ballantyne *et al*, 2004). Inhibition of coagulation with heparin has even been found to increase cancer survival (Ye *et al*, 1999). Thus, there is emerging evidence that the inflammation – coagulation interface dominantly contributes to many of the most common disease processes.

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