Chapter 2

Inflammation and Acute Phase Proteins in Haemostasis

Simon J. Davidson

Additional information is available at the end of the chapter

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1. Introduction

Inflammation is a very complex reaction to infection or injury the endeavour being to contain the infection and harm to a limited area. The process is associated with the activation of the coagulation system. To think that inflammation occurs and then leads to activation of the coagulation system is not quite true as there is much cross-talk between the two systems. This is a natural host response to cellular damage or infection however an overshooting of this cross-talk between coagulation and inflammation can lead to an exaggerated prothrombotic state and exacerbate the disease process. A wide range of inflammatory conditions such as infections, acute respiratory distress syndrome and SIRS (systemic inflammatory response syndrome) following major surgery e.g.cardiac surgery can lead to an uncontrolled inflammatory response and to profound disturbance of the coagulation system leading to an imbalance in the normal anticoagulated state of blood to that of a procoagulant state. When coagulation is compromised it can contribute to the pathogenesis of the inflammatory condition with deposition of fibrin within the microvasculature directly enhancing the inflammatory reaction. This in turn leads to a modulation of protein manufacture mainly via Liver hepatocytes in the upregulation and downregulation of at least twenty factors directly involved in blood coagulation. This process is controlled via cytokines and leads to the imbalance in what are called the haemostatic acute phase proteins. All of which puts the haemostatic system at an increased thrombotic potential [1, 2, 3, 4].

The haemostatic system maintains blood in a fluid phase under normal physiological conditions and provides a mechanism to prevent exsanguination upon vascular damage. Morawitz had created the 'classic' theory of blood coagulation in 1905 but it was Macfarlane who first reported the coagulation cascade as a biochemical amplification pathway of pro-enzyme-enzyme transformations in 1964 [5]. Davie and Ratnoff later the same year referred to it as a waterfall stepwise sequence of activation [6]. Macfarlane's idea that amplification of the cascade and acceleration of earlier stages of the pathway culminating in the conversion of

fibrinogen to fibrin was a major breakthrough in the understanding of how the coagulation factors interacted and forms the basis of what we understand today.

Platelets are the primary haemostatic plug when damage first occurs to a vessel. The multimeric protein von Willebrand factor mediates the adhesion of platelets to the site of vascular damage. The platelets bind to the matrix proteins exposed by the damage to the vessel wall, particularly collagen. These platelets are activated by small amounts of thrombin (~1nM) produced by tissue factor exposure at the site of vascular damage. The tissue factor binds factor VII that is rapidly activated. The tissue factor-VIIa complex subsequently activates factor X. The activated platelet provides binding sites for the coagulation enzymes, localising coagulation to the site of injury and prolonging activation of coagulation by protecting the enzymes from inhibition and inactivation [7]. Factor X is essential to this 'propagation' phase of coagulation. When bound to the platelet and activated via the VIIIa-IXa complex Xa is protected from inhibition by tissue factor pathway inhibitor and antithrombin.

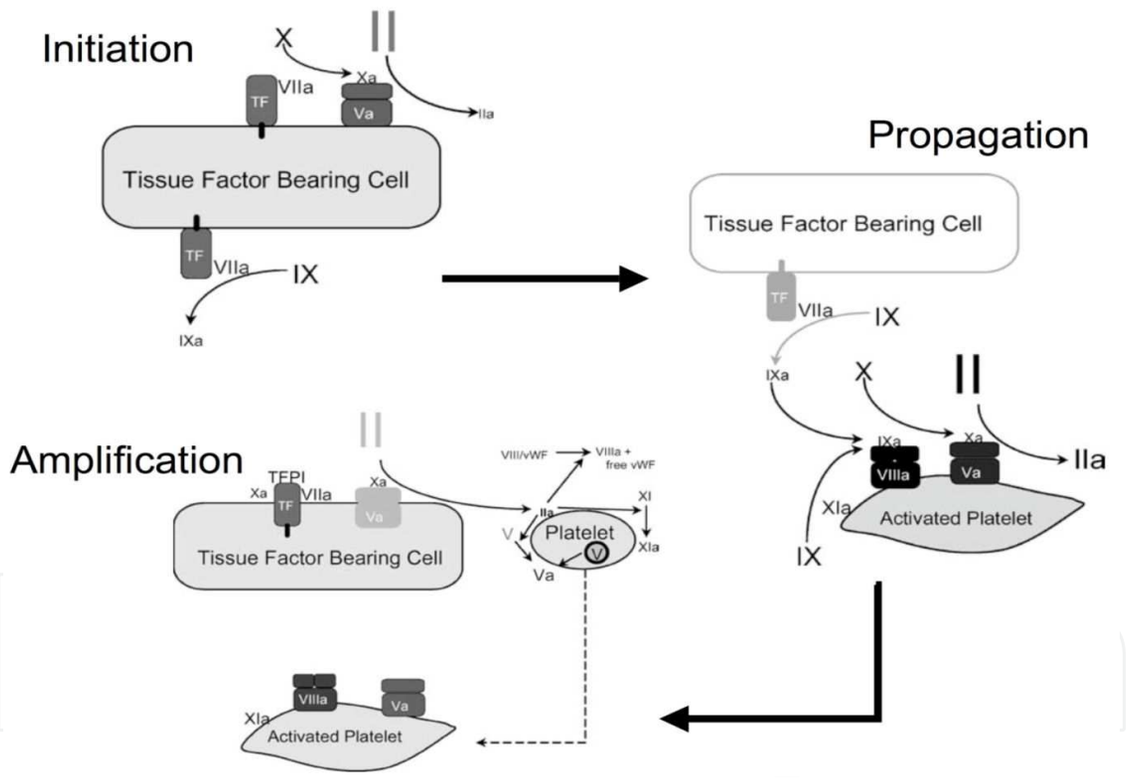

Figure 1. Cell based model coagulation. Reproduced with kind permission of Professor M.Hoffman

Tissue factor pathway inhibitor (TFPI) rapidly blunts this driving force of tissue factor-VIIa complex that initiates coagulation and generates the sudden burst of thrombin [8]. Once trace amounts of thrombin have been formed this is then able to activate factors V, VIII, IX and XI. This positive feedback mechanism ensures prolonged activation of the system with sufficient quantities of thrombin being produced to activate platelets, white cells, endothelial cells, and the protein C anticoagulant pathway and to continue producing thrombin.

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Coagulation activation yields proteases that not only interact with coagulation protein zymogens but also with specific cell receptors to induce signaling pathways that mediate inflammatory responses [9]. Many in vitro observations point to a role of coagulation proteases in upregulating the expression of proinflammatory mediators [10]. The most important mechanism by which coagulation proteases influence inflammation is by binding to protease activated receptors (PARs), of which four types (PAR 1 to 4) have been identified, all belonging to the family of transmembrane domain, G-protein–coupled receptors [11]. Tissue factor is also a potential mediator of intracellular signaling of established inflammatory pathways, functioning as an intermediate for factor VIIa–induced activation of mitogen-activated protein kinases and calcium signalling [12]. It is tissue factor that binds to factor VII and drives thrombin generation leading to fibrin formation. Tissue factor is an integral membrane protein normally separated from blood by the vascular endothelium. Tissue factor is expressed in the vascular adventitia in astroglial cells. It also appears in tumour cells where it appears related to their metastatic potential. All of this activation of coagulation increases in some procoagulant factors (fibrinogen and fator VIII) with reduced fibrinolytic response and dampening of the natural anticoagulant potential have a profound effect on mortality.

Thrombin plays many parts (Figure 2) and with the anticoagulant protein, activated protein C, can activate specific cell receptors on mononuclear cells and endothelial cells which can affect cytokine production and inflammatory cell apoptosis.

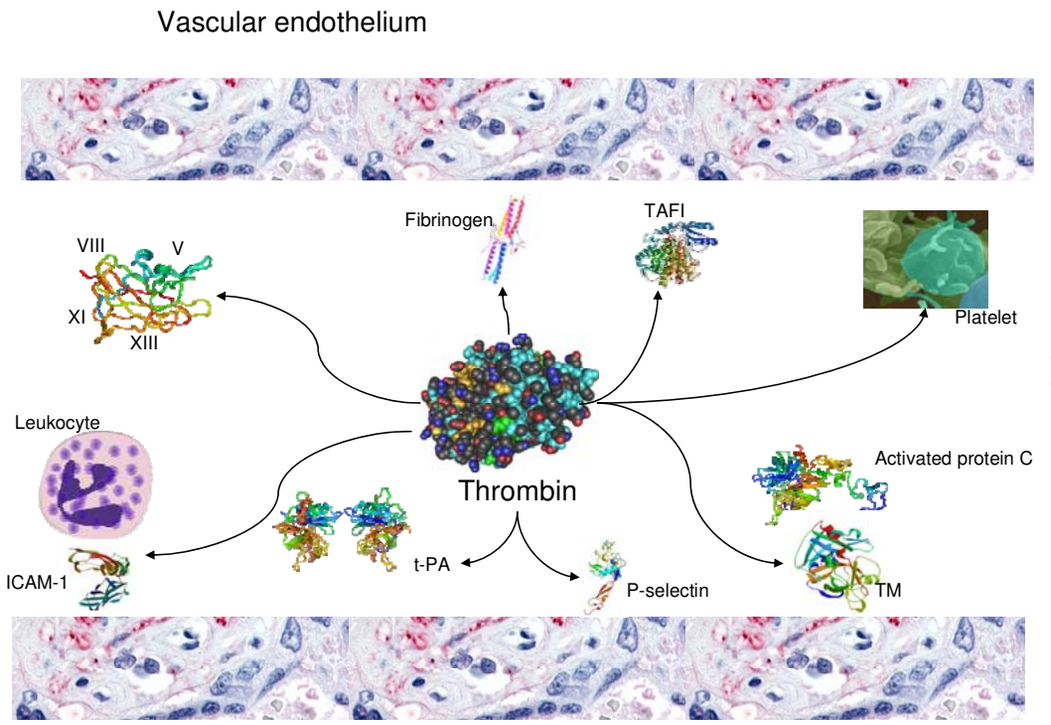

Figure 2. Thrombins role in activating some of the components of coagulation and inflammation. Coagulation factors V, VIII,XI and XIII, TM – thrombomodulin, TAFI – thrombin activatable fibrinolytic inhibitor, t-PA – tissue plasminogen activator, ICAM-1 intracellular adhesion molecule 1

2. Initiation of the pro-inflammatory response

Pathogen recognition receptors, Toll receptors (TLR), are essential in the host defence against pathogens. Platelets express these pattern recognition receptors involved in innate immunity. TLR's recognise microbial structures that are conserved among species. TLR1,2,4 6,8 & 9 all appear on platelets and form one of the many bridges between inflammation and coagulation. TLR's are responsible for LPS induced thrombocytopenia [13]. Platelets express TLR – pattern recognition receptors involved in innate immunity. TLR's are able to recognise danger associated molecular patterns (DAMPs). For example fibrinogen that is released during inflammation is a DAMP and further enhances the proinflammatory response through TLR4. It is via TLR and DAMPs that induction of caspase-1 activation which causes the processing of the proinflammatory response through various cytokines [14]. Figure 3

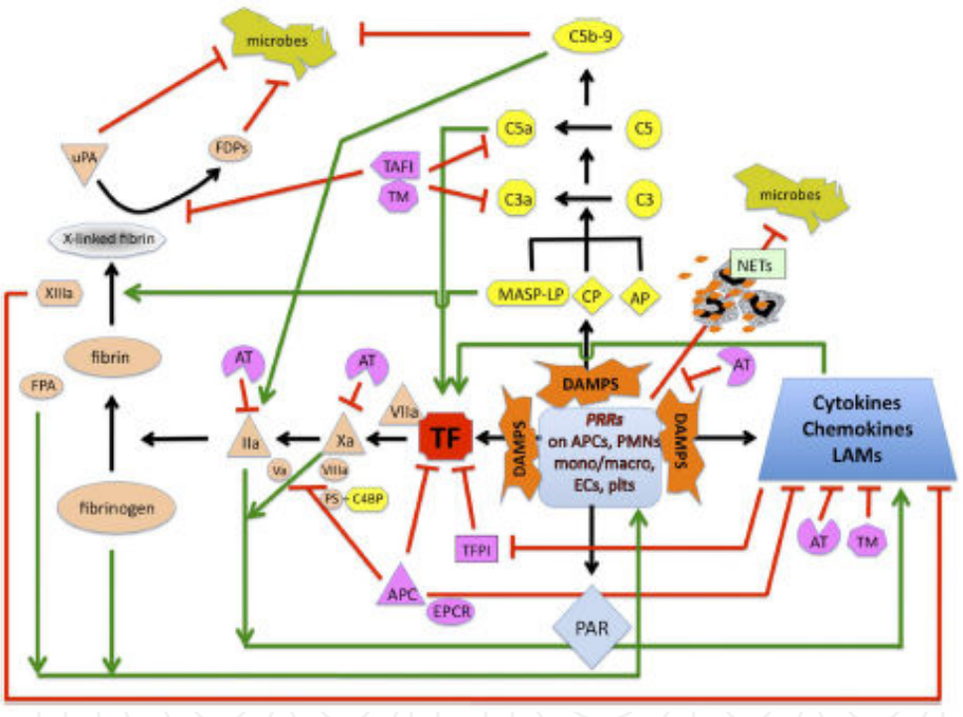

Figure 3. Cross-talk between coagulation and innate immune pathways in response to DAMPs (danger associated molecular patterns); DAMPS from invading pathogens or damaged host cellsare recognised by pathogen recognition receptors (PRRs) on antigen presenting cells, neutrophils, monocytes, macropahges, endothelial cells and platelets. This results in tissue factor exposure sustained by cytokines and chemokines with proinflammatory and opsonic properties and associated with increaased expressionof leukocyte adhesion molecules. In parallel DAMP-induced complement activation via any of the complement activation pathways leads to generation of the complement factors C3a and C5a and the membrane attack complex C5b-9. C5a feeds back to promote further tissue factor expresssion and C5b-9 also supports generation of thrombin. Green lines indicate increase in response, red lines indicate supression. Reproduced with kind permission of Dr EM Conway.

Cytokines are small molecules that have relatively short half-lives of between minutes and hours but play a pivotal role in the inflammatory response. The most important cytokines

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involved in regulating the coagulation response during inflammation are IL-6, TNF-alpha, IL-8, MCP-1 and IL-1 [15].

Acute phase proteins are released as mediators of the inflammatory cascade as a chemical and cellular response to injury. They increase rapidly in plasma in response to a inflammatory insult. Some acute phase proteins increase transiently (C-reactive protein) while others have a more sustained elevation (Haptoglobin) [16].

The inflammatory response to surgery, atherosclerosis, infection and cardiovascular disease has a profound effect upon the haemostatic system, including fibrinolysis. The cytokines interleukin 1β (IL-1β) and interleukin 6 (IL-6) modulate the production and suppression of many of the coagulation enzymes formed by the Liver.

The effect is to make the endothelium and whole coagulation system more procoagulant. Figure 4

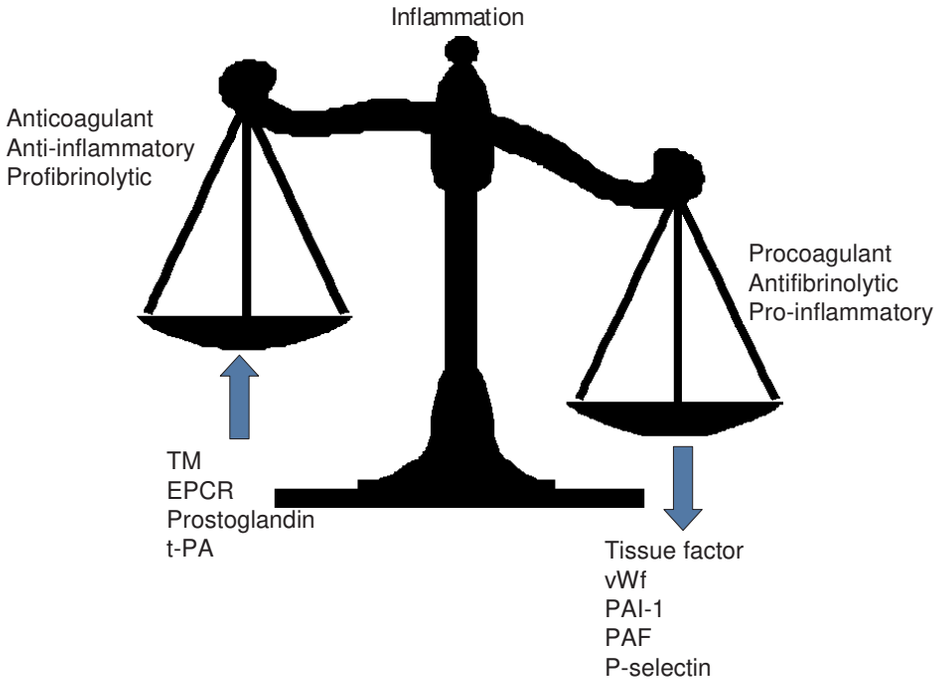

Figure 4. Inflammatory drive to a procoagulant state following endothelial stimulation.

3. Coagulation acute phase proteins

An acute-phase protein has been defined as one whose plasma concentration increases (positive acute-phase proteins) or decreases (negative acute-phase proteins) by at least 25 percent during inflammatory disorders [16]. The changes in the concentrations of acute-phase proteins are due largely to changes in their production by hepatocytes. Although

the mechanism by which the liver processes the stimulation to increase and decrease protein production may be different in different forms of inflammatory insult e.g. sepsis and chronic inflammation [17].

Proteins whose plasma concentrations increase with inflammation	Proteins whose plasma concentrations decrease with inflammation
Fibrinogen	Factor XII
Factor VIII	Antithrombin
Protein S	Histidine rich glycoprotein
Plasminogen activator inhibitor PAI-1 C4b-binding protein	Thrombomodulin
	Endothelial protein C receptor
Urokinase	Thrombin activatable fibrinolytic inhibitor TAFI?
α 1 Antitrypsin	Protein C (? No change/decrease)
α 2 Macrogloulin	
von Willebrand factor	
C1-esterase inhibitor	
C-reactive protein	
Thrombopoietin	
Thrombin activatable fibrinolytic inhibitor (TAFI)?	

Table 1. Acute phase proteins that directly affect haemostasis

4. Coagulation proteins whose plasma concentrations increase during inflammation

4.1. Fibrinogen

Fibrinogen is a soluble glycoprotein synthesised in the Liver with a normal plasma concentration of 2 – 4 g/L and half life of 4 days. When the coagulation cascade is activated fibrinogen is the final substrate in the formation of a clot being converted to its insoluble fibrin form. Thrombin cleaves fibrinogen releasing fibrinopeptide A and B forming fibrin monomers which have exposed polymerisation sites on the fibrin molecule. Thrombin activates factor XIII which cross-links these fibrin fibrils increasing clot strength and rendering them more resistant to proteolysis [18]. Fibrin creation is required with platelets (stimulating platelet aggregation by binding to the glycoprotein IIb/IIIa platelet membrane receptor) to repair any breach in the vascular integrity and prevent haemorrhage. This process is not left unchecked and is regulated via the fibrinolytic system (plasmin production) to prevent excess fibrin accumulation at the site of damage despite the procoagulant signalling drive. The local production of plasmin is regulated via two plasminogen activators, tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA) which under normal physiological conditions keeps this fibrin matrix production and its lysis tightly controlled [19].

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During an inflammatory reaction fibrinogen can increase in the order of 2-3 fold and this will significantly increase blood viscosity and cause some degree of red cell aggregation as well as transform vascular pathologies such as atherosclerotic plaques. Fibrinogen by means of increasing the production of endothelin-1, is also capable of directly inducing vasoconstriction [18].

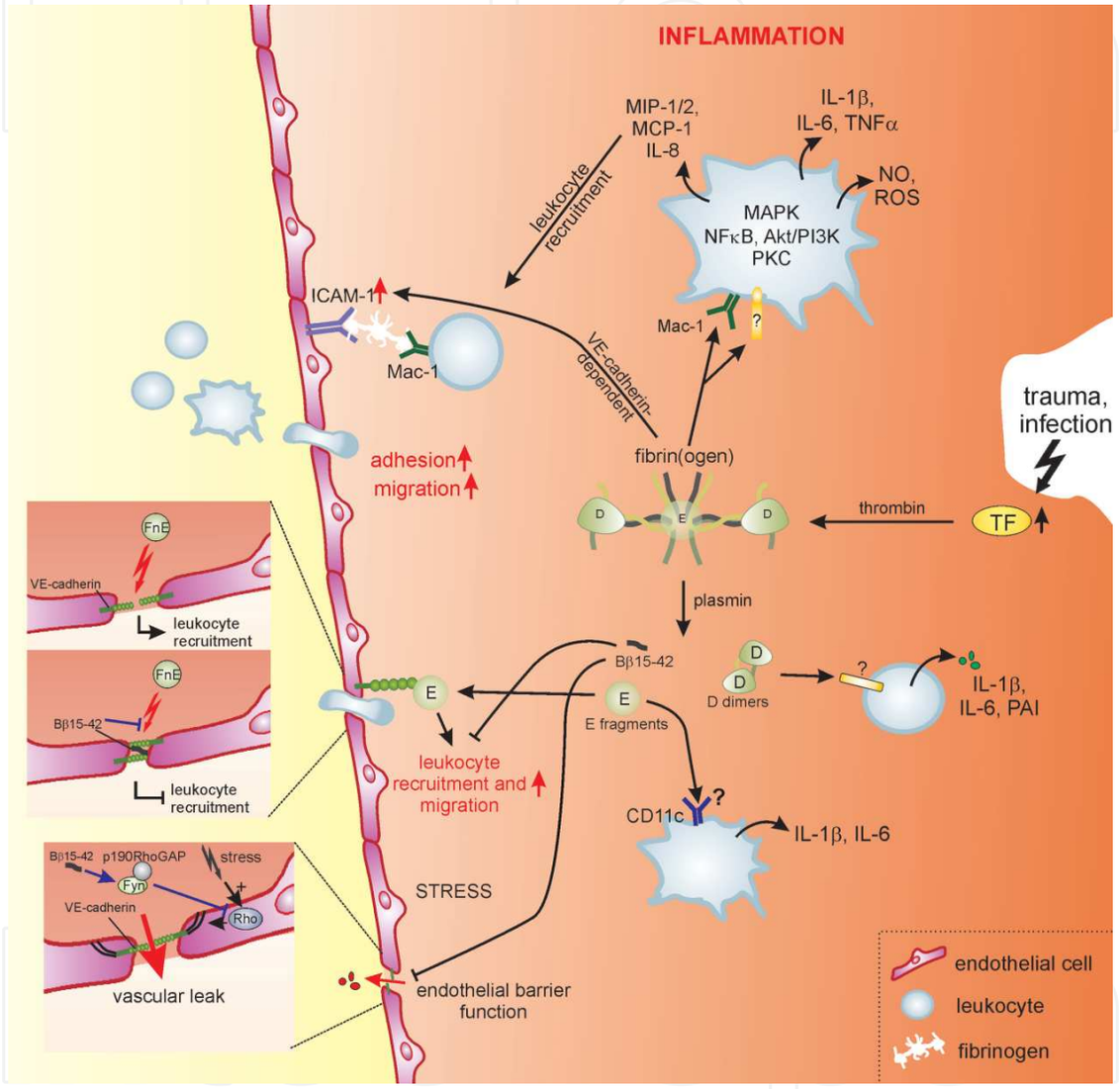

Figure 5. Fibrinogen and fibrin modulation of inflammation. Fibrin(ogen) modulates the inflammatory response by affecting leukocyte migration but also by induction of cytokine/chemokine expression mostly via Mac-1 signalling. NO-nitric oxide, Mac-1 Macrophage antigen 1, PI3K Phosphoinositide-3-kiniase, TNF tumor necrosis factor. Reproduced with kind permission of Prof. K Zacharowski

There is good evidence that fibrinogen has a function in regulation of the inflammatory response as is seen in the increased concentration of fibrinogen associated with atherosclerosis and cardiovascular risk. Fibrin(ogen) also participates in activation of vascular cells and regulation of the inflammatory response via the ability to bind to and activate a number of immune cells. The cellular ligand binding of fibrinogen is totally distinct to any coagulation

function. Fibrin(ogen) can bind to the integrin receptor Mac-1 which is found on many myeloid cells including monocytes and neutrophils and also T cells. The Mac-1 integrin is involved in phagocytosis, adhesion, migration through the endothelium as well as apoptosis and degranulation (Figure 5). Binding of fibrinogen to Mac-1 also induces production of cytokines IL-1β, IL-6 and TNF-α potentiating the inflammatory response, as many acute phase proteins do [20].

When fibrin is laid down in the microvasculature bed this enhances local and systemic inflammation via expression of proinflammatory mediators. Fibrin(ogen) increases mRNA concentration and induces synthesis of the proinflammatory cytokines IL-6 and TNF-α in monocytes and macrophages which is induced by chemokine expression, macrophage chemotactic protein (MCP-1) and macrophage inflammatory protein (MIP-1 and 2). The Toll receptor TLR 4 facilitates this fibrin(gen) chemokine expression signalling [20].

Breakdown products of fibrin, D-dimers stimulate monocytes to release the following IL-1, IL-6 and PAI-1 and fibrin degradation products also induce C-reactive protein production.

4.2. Factor VIII

Factor VIII is a procoagulant whose deficiency leads to classical haemophilia and a bleeding diathesis [21]. Factor VIII once activated by small amounts of thrombin becomes a co-factor for activated factor IXa in the formation of the tenase complex whereby these catalysts convert factor X to its activated form Xa. A number of publications have shown raised factor VIII to be linked with venous thromboembolism and increased thrombin generation [22, 23]. This increase in factor VIII has been linked with an increase in basal inflammatory reaction. Factor VIII increased production is mediated by the cytokine IL-6, however it is debateable as to how much a persistently raised factor VIII is responsible to inflammation by some [24]. Post operatively particularly after cardiac surgery factor VIII can be raised by as much as 2-3 fold.

4.3. Protein S

Protein S is a co-factor for protein C and produced mainly in the hepatocyes but also endothelial cells and megakaryocytes. Once protein C has been activated by the thrombin thrombomodulin complex, which is augmented by the endothelial protein C receptor, activated protein C dissociates from the endothelial protein C receptor and binds with protein S. This complex is then able to inhibit factors Va and VIIIa, protein S enhancing the reactive cleaving of specific sites by APC on factors V and VIII by anything up to 20-fold. At any one time only approximately 40% of circulating protein S is free and able to participate in this reaction. The remaining 60% of circulating protein S is bound to the complement regulator protein C4B-binding protein and lacks the co-factor functionality in this reaction. Consequently the protein S-C4BP complex limits the functionality of activated protein C in its anticoagulant role. Therefore protein S plays an important role in the regulation of thrombin generation although it being a risk factor for venous thrombosis in deficient patients remains unclear and the odds risks have varied between 0 – 11.5 fold in protein S deficient cases.

Protein S has an accelerating role in APC mediated PAI-1 inhibition thereby promoting clot lysis and a possible inhibition of activation of thrombin activatable fibrinolytic inhibitor [25].

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Protein S has been shown to increase during inflammation. This may in part be due to counterbalancing the procoagulant drive of the coagulation system in these circumstances by providing more anticoagulant effect via the protein C pathway. It may also have to do with other non-anticoagulant actions it has via its binding with C4BP (see below). Certainly inhibition of protein S in in-vivo models of bacteraemia have shown to provoke the cytokine response and what was a non-lethal injected dose of *E.coli* in baboons resulted in a lethal reaction [26].

4.4. C4b-binding protein

The complement binding protein C4BP is a co-factor to a serine protease in the degredation of C4 in the classical complement pathway and providing it has been suggested protection from inflammation at a cellular level. The C4BP molecule has a central region with seven α chains and one β chain emanating from it each with distinct ligand binding regions. It is on the β chain side arm that the protein S binding region resides and in normal human plasma ~80% of C4BP is found as this β chain form. C4BP is an acute phase protein and its levels can increase to 400% of normal [25]. However this is mainly of the C4BPα form so the Protein S-C4BP complex concentration is not generally affected. As so much protein S is normally bound to C4BP and the concentration of the free anticoagulant active co-factor protein S level quite tightly controlled is there a physiological role for the PS-C4BP complex? It has been suggested that protein S forms one of the bridges between coagulation and inflammation. As protein S has a high affinity to bind to negatively charged phospholipids it can bring C4BP into close proximity to these sites allowing controlled complement activation at areas of vascular damage where coagulation is activated. This has been linked to apoptosis and the complement system allowing rapid clearance of apoptotic cells from the site of damage by macrophages but more probably limiting or inhibiting further complement activation via necrotic cells [27, 28].

4.5. Plasminogen activator inhibitor PAI-1

The fibrinolytic system is the opposite of the coagulation system. It limits clot formation to the site of injury and breaks down existing clot by an enzyme cascade. It is also, like haemostasis, linked to inflammation with acute phase proteins that increase and others that are decreased. Plasminogen provides the fibrinolytic potential and when converted to its active form plasmin is able to bind to fibrin and begin its degradation. Plasminogen is activated by two activators, tissue plasminogen activator (t-PA) and urokinase tissue plasmingen activator (u-PA). These in turn are inhibited by plasminogen activator inhibitor 1 and 2 (PAI-1, PAI-2) and plasmin is inhibited by α-2-antiplasmin. Of these inhibitors PAI-1 is a positive acute phase protein and increases during inflammation. The cytokine tissue necrosis factor

(TNF-α) suppresses finbrinolysis by down regulating t-PA expression in endothelial cells while production PAI-1 then in turn PAI-1 inhibits TNF-α is inhibited by PAI-1. It has also been shown that by inhibiting TNF-α plasma levels of PAI-1 decrease [19].

The increase in PAI-1 levels is again a driver of the haemostatic system towards a more prothrombotic state. PAI-2 will not be discussed here but for interested readers they are directed to the following publication [29].

4.6. Urokinase

Urokinase plasminogen activator (u-PA) is a multifunctional serine protease. It has been shown to act as both a signalling ligand and a proteolytic enzyme. As an acute phase protein its increase has been found in a number of metastatic carcinomas and this has led to a link in its role of tumor growth and cellular expansion in conditions such as cardiac fibrosis and atherosclerosis [30]. As well as displaying involvement in tissue remodelling u-PA also appears to regulate macrophage activation and function. The macrophages not only synthesis and release u-PA but also have receptors for the u-PA protease on their membranes. The excreted u-PA induces TNF-α synthesis and secretion. The u-PA molecule has also been shown to initiate inflammation via the release of IL-6 and IL-1β from monocytes and lymphocytes [31]. The u-PA and its receptor uPAR also mediate immune complex induced inflammation in the lung. This is achieved via generation of u-PA at the site of cellular inflammation with subsequent activation of its receptor uPAR. This then sets off cellular signalling with C5a/C5aR on the alveolar macrophages, recruitment of polymorphonuclear leukocytes and adequate TNF-α production [32].

4.7. α 1 Antitrypsin

The α-1-antitrypsin glycoprotein is a proteinase inhibitor synthesised and secreted in the hepatocytes of the Liver. It is one of two physiological inhibtors of activated protein C. It also has a profound anti-inflammatory effect in the lungs with concentrations during a inflammatory response reaching levels found in the plasma. The range of antiprotease activity seen in the lungs includes neutrophil elastase and plasminogen acitvators. If there is a deficiency of α-1-antitrypsin (as with the genetically abnormal SERPINA1 gene mutations) then neutrophil elastase provoked by infection and inflammation will unchecked breakdown elastin and destroy alveolar walls leading to emphysema [33].

4.8. Alpha-2-macroglobulin

Alpha-2-Macroglobulin (α-2M) is an ancient serine protease binding host or foreign peptides and particles, thereby serving as humoral defense barrier against pathogens in the plasma and tissues. In humans α-2M, interacts and captures virtually any proteinase whether self or foreign, suggesting a function as a unique "panproteinase inhibitor." In adult humans it provides somewhere between 10-25% of the overall anti-thrombin activity in plasma. It is also the primary inhibitor of thrombin in neonates and infants under 1 year of age until the Liver matures and begins producing sufficient Antithrombin to take over the role. At times of inflammation when antithrombin levels are low α-2M can become a ‘back-up’ thrombin inhibitor [34].

4.9. C1-esterase inhibitor

C1-inhibitor (C1INH) is a serpin and major inhibitor of the contact coagulation system that involves factor XII, kallikrein and kininogen. It is also an important regulator of complement activation inhibiting the first component of complement C1. Another important biological role of C1INH is vascular permeability regulation [35]. This is well illustrated in patients who suffer from hereditary angioedema where there is a deficiency of the C1INH activity. As it now seems factor XII plays a substantial part in the formation of thrombin during sepsis and inflammation where neutrophil extracellular traps are present that release polyphosphate that in turn activate factor XII it would appear normal for C1INH to act as a positive acute phase protein in limiting this activation as well as its complement inhibitory role. A rapid appearance of C1INH-factor XIIa complexes is reported during sepsis with a sharp fall in the C1INH activity wherein the inhibitory function of α-2Macroglobin becomes more important in its kallikrein inhibiting role [36].

4.10. von Willebrand factor

The von Willebrand factor (VWF) is a multimeric protein that mediates the adhesion of platelets to the exposed subendothelium at the site of vascular injury. It also serves as a carrier protein for the labile coagulation factor VIII. VWF is synthesised in and stored in the Weibal-Palade bodies of endothelial cells and megakaryocytes/platelets. Endothelial cells release VWF when activated or stimulated. These VWF molecules are ultralarge and hyperactive, proficient in binding to platelets via the glycoprotein Ib-X-V complex without any external activation. Normally the ultralarge VWF multimers are cleaved into smaller less active multimers before being released into plasma. Cleavage is performed by ADAMTS-13 (a disintegrin and metalloprotease with thrombospondin motif). A deficiency of ADAMTS-13 leads to a thrombotic thrombocytopenic purpura a thrombotic microangiopathy. Increased plasma levels of VWF have been reported in a number of disease states including coronary artery disease, autoimmune disease, trauma and infections. A common underlying process in all of these is inflammation. There appears to be a complex interaction between the common inflammatory cytokines IL-6, IL-1β and TNF-α the release and cleavage of the VWF multimers by ADAMTS-13.

Release of VWF multimers from endothelial cells occurs in a dose dependent fashion when stimulated with IL-8 and TNF-α and IL-6 inhibits cleavage of the ultrlarge multimers. This demonstrates that inflammatory cytokines interfere with the equilibrium of release and rate of cleavage of the ultralarge VWF multimers.

This increases prothrombotic risk in this setting as most of the positive acute phase proteins have a propensity to do creating a procoagulant environment. It is of interest to note that IL-8 resides in the Weibel-Palade bodies of the platelet the same place as VWF and is involved in the platelet leukocyte aggregation and it is TNF-α that activates endothelial cells releasing IL-8. The inhibition by IL-6 of ultralarge multimer cleavage by ADAMTS-13 may be by way of synthetic and secretion inhibition. In overwhelming sepsis there is also probably an element of exhaustion of metalloprotease activity [37].

4.11. C-reactive protein

C-reactive protein (CRP) is a pentameric molecule that increases several hundred fold following an inflammatory stimulatory response this being primarily due to IL-6 stimulation of production of CRP in the hepatocytes of the Liver. CRP amplifies the host defence mechanism by activation of complement via C1 and stimulation of macrophages. CRP also upregulates tissue factor expression on monocytes [38] and induces release of PAI-1 thereby downregulating fibrinolysis [39] promoting a procoagulant state. The pentameric CRP is thought to be directly proinflammatory at high concentration like those of sepsis or major surgery but more subtle inflammatory reactions take place when monomeric CRP is released from the pentameric form. This appears to be driven by activated platelets revealing new lipid messenger sites; lysophosphatidycholine (LPC) that bind and dissociate the pentameric form of CRP to the monmeric form [40].

During sepsis when disseminated intravascular coagulation (DIC) occurs this has been found experimentally to co-inside with formation of a calcium-dependent complex between CRP and very low density lipoprotein (VLDL). The VLDL molecular makeup is different in septic patients to normal controls due to a deficiency of phosphatidylethanolamine. This CRP-VLDL lipid raft increases the procoagulant effect through an increase in prothrombinase activity. The CRP-VLDL complex exists in vivo and it has been postulated that is has a pathogenic role in disseminating the intravascular coagulation [41].

5. Coagulation proteins whose plasma concentrations decrease during inflammation

5.1. Antithrombin

Antithrombin (AT) is one of the major natural anticoagulats inhibiting thrombin, factor Xa, IXa and factor VIIa bound to tissue factor. Inhibition of factors Xa, IXa and the VIIa-tissue factor are accelerated via the endothelial cell heparin like proteoglycans. The importance of this anticoagulant pathway is highlighted when AT levels are low, either congenitally or acquired, the risk of thrombosis is significantly increased.

AT has been shown in-vitro using HUVEC cells and IL-6 to act as a negative acute phase protein [42]. Other mechanisms that can reduce AT function during an inflammatory response include increased consumption from activation of haemostasis and increased degradation by proteolytic enzymes (elastase released from activated neutrophils). Furthermore inflammatory cytokines can induce a reduction in the production of glycosaminoglycans such as heparin and chondroitin sulphate on the endothelial cell which may contribute to the impaired function of AT due to GAGs acting as physiological heparin-like cofactors promoting the anticoagulant anti-thrombin activity of AT [43, 44].

AT can indirectly act as an anti-inflammatory molecule by directly inhibiting thrombin reducing its inflammatory properties of haemostatic and complement activation, leukocyte,

endothelial and platelet activation. AT also appears to directly act as an anti-inflammatory agent by binding with leukocytes receptors blocking their communication with endothelial cells and limiting their adhesion and migration [45]. In animaI models it has also been shown that AT induces the release of prostacyclin from endothelial cells. The prostacyclin is a platelet inhibitor and abrogates neutrophils adhering to endothelial cells both of which contribute to the inflammatory response [46].

In addition AT can also modulate cellular receptor expression by downregulating the expression of CD11b/CD18 on leucocytes which bind factor X aiding its activation [47].

AT is also able to decrease expression of tissue factor and IL-6 expression on monocytes and endothelial cells reducing the inflammatory drive [48].

5.2. Histidine rich glycoprotein

Histindine rich glycoprotein (HRGP) is a single polypeptide chain protein that is synthesised in the Liver found in plasma and on the surface of leukocytes, monocytes and the α-granules of platelets. HRGP has been shown to bind to a number of molecules including, haem, Zn^{2+}, plasminogen, fibrinogen, IgG, complement and factor XIIa (not the inactive zymogen) [49]. HRGP exerts some degree of innate immune response by exhibiting antimicrobial activity to some organisms and removal of necrotic cells by binding to free decondensed DNA (polyphosphate).

HRGP behaves as a negative acute protein during inflammation [50]. This is of interest as fibrinogen one of its primary ligands is increased. Possibly more importantly is the fact that HRGP strongly inhibits polyphosphate induced factor XII autoactivation via increased Zn^{2+} levels in response to local platelet activation, showing it to be a significant modulator of factor XII activation during sepsis and inflammation [51]. Because of its negative acute phase nature this will allow increased activation of factor XII in these circumstances potentiating the pro-inflammatory and pro-coagulant condition.

5.3. Thrombomodulin

Thrombomodulin (TM) is present on endothelial cells of the entire vasculature and is a transmembrane protein with epidermal growth factor (EGF) repeating molecules that stick out of the plasma membrane and provide binding and activation sites for a number of molecules. There is a high affinity binding site for thrombin on EGF domain 5-6 from which protein C is activated at EGF domains 4-6. Thrombin activatable fibrinolytic inhibitor is activated by thrombin at EGF sites 3-6 [52]. When thrombin binds and complexes with thrombomodulin on the cell membrane its ability to activate protein C increases by greater than 1000-fold. Activated protein C anti-inflammatory effects are discussed later in this chapter, see below. Once thrombin has bound to TM it becomes quickly neutralised by its natural serine proteases inhibitors antithrombin, heparin co-factor II and protein C inhibitor and therefore activation of protein C also ceases.

The effect of inflammation on TM is to decrease its presence on the endothelial cell surface by its cellular internalisation by endocytosis via TNF-α. This creates a site where coagulation can

take place as the anticoagulant barrier has been removed and it may also stimulated further inflammatory response [43]. CRP has also been shown in experimental conditions using human coronary artery endothelial cells treated with CRP in a dose and time dependent manner to reduce messenger RNA levels of TM [53]. TM also provides anti-inflammatory protection from complement activation by enhancing inactivation of C3b and by promoting activation of thrombin-activatable fibrinolysis inhibitor that inactivates complement anaphylatoxins C5a and C3a [54]. Others have shown that thrombin-activatable fibrinolysis inhibitor activation via TM is attenuated by platelet factor 4 released from activated platelets [55].

5.4. Endothelial protein C receptor

The thrombomodulin-thrombin conversion of protein C to its activated form is facilitated by the endothelial protein C receptor (EPCR). The EPCR is another endothelial cell transmembrane protein located in close proximity to the thrombomodulin molecule and with high affinity binding for protein C. EPCR as well as binding protein C also binds its activated form and via membrane lipid rafts they complex with PAR-1 [56]. The EPCR-activated protein C molecule activates PAR-1 in a different way to thrombin which allows it to signal through a potent Gi protein pathway by which anti-inflammatory pathways are stimulated within the endothelial cell [57]. C-reactive protein has also been shown, as in the case of thrombomodulin, under experimental cell culture conditions to down regulate EPCR.

5.5. Thrombin activatable fibrinolytic inhibitor

Thrombin activatable fibrinolytic inhibitor (TAFI) is a basic carboxypeptidase identical to the plasma procarboxypeptidases B, U and R. TAFI is activated by thrombin and plasmin although the most efficient activator is the thrombomodulin-thrombin cellular membrane bound complex. TAFI inhibits fibrinolysis by cleaving lysine residues from fibrin which restricts the binding of tissue plasminogen activator to these sites and enhancing the plasminogen conversion to plasmin potentiating further fibrin breakdown [58]. TAFI certainly modulates the balance between coagulation and fibrinolysis but it appears its linking role with inflammation still needs to be fully elucidated. For instance TAFI has been shown to be a positive acute phase protein in mice [59] but in the same year Boffa et al interestingly showed that IL-6 administered to cultured HepG2 cells resulted in a 60% decrease in TAFI mRNA [58]. Subsequently TAFI has been shown to be raised in experimental endotoxemia [60]. Its anti-inflammatory properties are also downregulated by platelet factor 4 which is release from activated platelets and endothelial cells during activating stimuli and inflammatory insults which prevents TAFI activation by binding to the thrombin-thrombomodulin complex thereby preventing TAFI's inactivation of the complement anaphylatoxins C5a and C3a [55].

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6. Other major components of the acute phase response that affect haemostasis

6.1. Protein C

The protein C pathway is known to be an important anticoagulant system with patients deficient in protein C being at risk of thrombosis or in its homozygous form purpura fulminans. Activated protein C inhibits factors V and VIII this being supported by the activation of protein C by thrombin bound to thrombomodulin on the endothelial cell surface. As well as acting as an anticoagulant activated protein C is also able to inhibit PAI-1. The anticoagulant and antifibrinolytic aspects of the protein C pathway have been elucidated and well described although there still appears much to learn from the interaction of protein C during the inflammatory response.

It is unclear from studies of the acute phase proteins whether protein C acts as a positive or negative acute phase protein. Most studies show it to have no change in concentration during an inflammatory response or its plasma concentration to decrease. A decrease in protein C could be attributed to consumption as well as a cytokines limiting the natural anticoagulant response. Activated protein C also confers a cytoprotective, anti-inflammatory, anti-apoptosis and endothelial barrier stabilisation effect when active [61].

Activated protein C signals its anti-inflammatory effects mainly via PAR-1 pathways whereby following Gi signalling and sphingosine-1-phosphate production there is improvement in endothelial cell barrier function [62].

Transcriptional profiling studies using cell cultures of human umbilical vein endothelial cells (HUVECs) have demonstrated that recombinant human activated protein C can regulate endothelial cell gene expression linked to inflammation and cell survival [63]. The activated protein C suppresses NFκB a cell nuclear transcription factor, and by reducing its expression and function this in turn causes inhibition of cytokine signaling [64].

6.2. Cytokines

Cyotkines are a superfamily of molecules involved in cell signalling many of which are increased greater than 1000-fold during an inflammatory insult. Cytokines such as interleukin (IL)-6, but also platelet-derived growth factor and monocyte chemoattractant protein (MCP)-1 are capable of stimulating tissue factor expression in mononuclear cells. Tissue factor being the initiator of thrombin generation and fibrin formation.

IL-6 is a multifunctional cytokine that is induced in many disease states such as sepsis, endotoxaemia and in-vitro after administration of tumor necrosis factor (TNF). Several studies have suggested IL-6 to be a potential mediator of endotoxin induced coagulation activation. This has been validated by treatment of chimpanzees with a monoclonal anti-IL6 antibody that ablated the activation of coagulation [65]. Cytokines are pivotal in providing a means of cross talk between inflammation and coagulation [66].

Inflammation and coagulation can not be considered as two separate processes because there are several interlocking points making them a unique defensive host reaction. The endothelium is one of the major links between the two since damaged endothelium during inflammation represents a surface where proteins involved in both coagulation and fibrinolysis and the development of inflammation are expressed. Cytokines down regulate the surface receptor thrombomodulin and the activation of protein C but at the same time increase the expression of tissue factor. Platelets adhere to these sites of vascular damage and when activated also release several cytokine mediators of inflammation, adhesion molecules and growth factors including IL-1β, CD40 ligand, vitronectin and RANTES [13], [67].

In addition to the inflammatory cytokines like interleukin 1 and TNF, infection per se can trigger the release of neutrophil extracellular traps (NETs). Which in turn release cytokines and microparticles exacerbating the acute phase haemostatic response [68].

Cytokines also upregulate the complement system activated C5b9 complexes can assemble clot promoting membrane phospholipids, TNF-α downregulates thrombomodulin and vascular heparin sulphates promoting a procoagulant environment. The net effect of this is to further lessen the inhibitory mechanisms that control thrombin generation.

6.3. Platelets

Platelet numbers increase following surgery, trauma and sepsis following a inflammatory response. Both IL-6 and thrombopoetin stimulate the production of platelets [69]. This reactive thrombocytosis can last up to13 days post surgery and may responsible for PAF adverse events in liver reperfusion and thrombotic complications post cardiac surgery. It appears that thrombopoietin is an acute phase reactant but not uniquely responsible for the rise in the platelet count during a reactive thrombocytosis but is probably aided and abetted by IL-6 [70].

Platelets release CD40 ligand which induces tissue factor expression and increases inflammatory cytokines IL-6 and IL-8. (Esmon CT). The CD40 ligand is a transmembrane protein related to TNF-α which was originally identified on stimulated $CD4^+$ T cells. The interaction of CD40 on T and B cells is integral to the development and function of the humoral immune response. It is now known that CD40 ligand is found on many cells including macrophages, endothelial cells and platelets. Upon activation platelets express CD40L within seconds. As wtih TNF-α and IL-1 the CD40L on platelets induces endothelial cells to exude cytokines and up regulate the expression of adhesion molecules. This all increases the general recruitment of leukocytes to the site of injury. Platelets therefore directly initiate the inflammatory response at the vessel wall [14]. Figure 6

The concept that platelets play a key role in the host defence and inflammatory response has taken longer to realise mainly due to their role in primary haemostasis. This new role of platelets as immune effector cells is enhanced by the finding that platelets directly interact with microbes and bacteria.

Platelet activation along with procoagulant events and fibrin formation seem crucial for the containment and killing of bacteria. Platelets have recently been shown to induce the formation of neutrophil extracellulat traps (NETs). NETs are lattice arrangements of decondensed nuclear

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chromatin which are laced with histones that have antimicrobial properties. It also appears that this may be an 'overshooting' of the hosts defence mechanism with further platelet activation, thrombosis and endothelial cell injury [71], [72], [73].

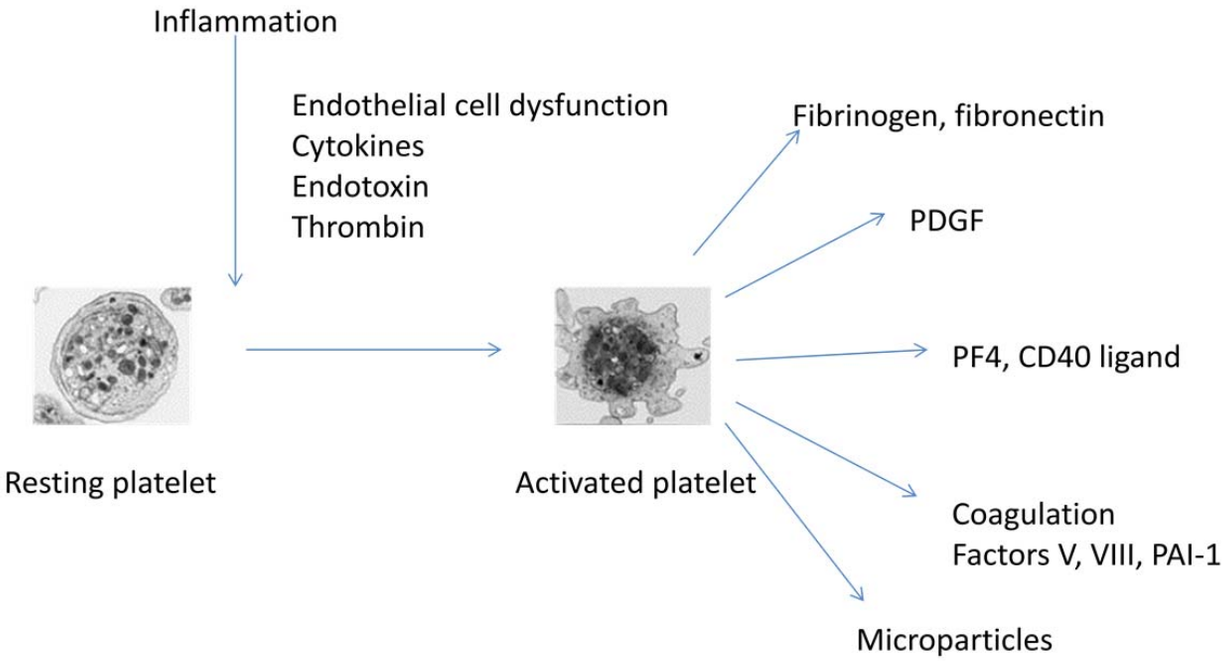

Figure 6. Inflammation and platelet activation. Platelet derived growth factor – PDGF, platetlet factor 4 – PF4.

6.4. Endothelium

Endothelial cells in the vasculature are actively involed in haemostasis providing an anticoagulant surface preventing activation of the coagulation system. Endothelial cells produce elements with proinflammatory, procoagulant and antifibrinolytic properties as well as those with the opposite anti-inflammatory, anticoagulant and profibrinolytic properties. When endothelial cells are activated or damaged they release into the local surroundings procoagulant components such as von Willebrand factor (a platelet binding factor and a carrier protein for factor VIII) and thromboxane A2 (a platelet activator) and plasminogen activator inhibitor (PAI-1 a potent inhibitor of tissue plasmingen activator). The opposite is true of components that provide an anticoagulant surface in the milieu of the blood vascular barrier. Thrombomodulin, a thrombin binding transmembrane protein that switches thrombin from a procoagulant enzyme to one that activates protein C, a nautral anticoagulant that inhibits the activity of factors V and VIII, in internalised within the endothelial cell. Thrombomodulin is also cleaved from the endothelium by activated Neutrophils. Tissue factor is expressed on the cell surface along with adhesion molecules that mediate the interaction of neutrophils and platelets these included vascular cell adhesion molecule (VCAM-1), P and E selectin and intracellular

cell adhesion molecule (ICAM-1) all of which promote the inflammatory response. So the endothelium serves as an interface for the inflammatory response leading to local activation of the coagulation system, vasodilatation and pro-inflammatory state [3], [57].

6.5. Complement

Complement is part of the innate immune system and the effector of antibody mediated immunity. The biological functions of complement include the defence against infections and the clearance of immune complexes and apoptotic cells. The complement cascade is made up of approximately 30 proteins circulating in plasma and expressed on cellular surfaces. The complement cascade is activated via three pathways: classical, lectin and alternative. The classical pathway is initiated by the binding of C1q to antigen–antibody complexes. The lectin pathway is initiated via the binding of mannose-binding lectin or ficolins to sugars found at the bacterial cell wall. Both of these pathways lead to the formation of a C3 convertase. The alternative pathway is stimulated by spontaneous hydrolysis of internal thioester bonds within C3. C3a and C5a are anaphylatoxins and inflammatory mediators which are inhibited by TAFI.

Complement and coagulation are again two systems that cannot be viewed separately during an inflammatory response. Complement contributes significantly to the prothrombotic state during inflammation. The direct procoagulant activities of complement involve the activation of platelets via C3a and the C5b-9 membrane attack complex and the upregulation of tissue factor and PAI-1 expression on various cell types by C5b. Thrombin has also recently been identified as an activator of C5 linked to the coexistence of a C5 convertase enzyme [74], [75], [76].

It is of interest to note the complement factor C4B-binding protein is a positive acute phase protein that can increase greater than 400% during inflammatory states. The C4B-bp in normal circumstances binds the natural anticoagulant protein S. Protein S acts as a co-factor with activated protein C in the inactivation of factors V and VIII. It was thought that an increase in C4B-bp may increase the binding of circulating protein S, approximately 60% of protein S is normally bound leaving the other 45% free protein S to aid in the V and VIII inactivation. However this increase is restricted to the C4BPα+ form, which does not bind to PS. Therefore, the blood levels of the active free form of PS remain stable even during an acute phase response. The normal binding of protein S to C4B-bp probably allows this complex to bind via protein S high affinity for negatively charged phospholipids depositing it at area of cellular damage and limiting further apoptosis through complement activation as it will block C4b (see Protein S).

Author details

Simon J. Davidson

Department of Haematology, Royal Brompton Hospital, London, UK

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