

Complement and coagulation: strangers or partners in crime?

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The convergence between complement and the clotting system extends far beyond the chemical nature of the complement and coagulation components, both of which form proteolytic cascades. Complement effectors directly enhance coagulation. These effects are supplemented by the interactions of complement with other inflammatory mediators that can increase the thrombogenicity of blood. In addition, complement inhibits anti-coagulant factors. The crosstalk between complement and coagulation is also well illustrated by the ability of certain coagulation enzymes to activate complement components. Understanding the interplay between complement and coagulation has fundamental clinical implications in the context of diseases with an inflammatory pathogenesis, in which complement-coagulation interactions contribute to the development of life-threatening complications. Here, we review the interactions of the complement system with hemostasis and their roles in various diseases.

Complement and clotting – contributors to inflammation

Our rapidly increasing understanding of the biology of the complement system (Box 1) and hemostasis (Box 2) has indicated several interesting interactions that exist between these two entities. In the light of recent investigations, both systems can be viewed as partners in an inflammation that is aimed at stabilizing a living system that has encountered various disturbances to its homeostasis. The complement cascade is activated by the same stimuli that launch inflammation: for example, when the danger of infection is detected or the host tissue is damaged [1]. These situations are generally also associated with an increased propensity for blood clotting [2]. By contrast, trauma that causes injury to the vasculature and subsequent bleeding, which would usually activate blood coagulation, is also associated with a risk of infection and the activation of an inflammatory reaction [3]. Therefore, in most pathophysiological situations, it seems that the activation of both the complement and coagulation cascades

occurs simultaneously. This concurrent activation of hemostasis and inflammation is protective, and in many clinical circumstances it is beneficial for the host because an efficient response to pathogens or to stimuli initiating bleeding requires the synchronized activity of various biological effectors. For example, it is clear that the coagulation system has an important role in host–pathogen interactions and the responsiveness of the host to infection. The local formation of thrombi (blood clots in the lumen of intact blood vessels) in the microvasculature draining the site of microbial invasion provides a competent barrier that prevents the spread of bacteria into the circulation [4]. In fact, several bacterial strains have developed strategies involving the production and secretion of various fibrinolytic factors that enable the organisms to overcome these limitations [5]. It is important to note that complement and coagulation cascades are intended to act locally – that is, complement is activated at the site of infection and coagulation at the site of bleeding. However, when these cascades are activated systemically as the result of a failure of the relevant control mechanisms, the effects of this widespread reaction seriously threaten the host.

The common stimuli and similar pathophysiological situations that result in the activation of both cascades are not the only parallels between the complement and coagulation systems. At various levels of inflammatory or hemostatic responses, members of both cascades interact with each other directly, either through interactions with target cells or through processes involving inflammatory mediators other than complement effectors. These complex and reciprocal interactions also have a role in the pathogenesis of several diseases, and, in some situations, they lead to life-threatening complications, such as thrombosis (the formation of multiple thrombi) in systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS) [6], disseminated intravascular coagulation (DIC) [7] or multiple organ failure (MOF) [3]. A deficiency in the negative regulators of complement results in excessive complement activation, as observed in paroxysmal nocturnal hemoglobinuria (PNH) and atypical–familial hemolytic uremic syndrome (aHUS), and results in an increased tendency to develop thrombosis [8]. Although, in most

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Box 1. Complement activation and functions

Complement is not only a part of the innate immune system but also an effector of antibody-mediated immunity. The major biological functions of complement include the defense against infections, bridging innate and adaptive immunity, and the clearance of immune complexes and apoptotic cells. The system is composed of ~30 proteins circulating in plasma and expressed on cellular surfaces. Circulating components of complement are activated through three pathways: classical, lectin and alternative. The classical pathway is initiated by the binding of C1q to antigen-antibody complexes, whereas the lectin pathway begins with the binding of mannose-binding lectin (MBL) or ficolins to sugars presented at the bacterial cell wall. Both pathways lead to the formation of a C3 convertase. The alternative pathway is triggered by spontaneous hydrolysis of the internal thioester bond within C3 in the fluid phase, leading to the formation of C3-H₂O. Hydrolyzed C3, through the binding and subsequent activation of factor B, contributes to the formation of the alternative pathway C3 convertase. C3 convertases cleave C3 to C3a and C3b. C3b participates in the formation of the C5 convertase and functions as a bacterial opsonin, facilitating the phagocytosis of opsonized pathogens by macrophages and neutrophils. C5 convertase cleaves C5 to C5a and C5b. C3a and C5a, termed anaphylatoxins, are pleiotropic inflammatory mediators. C5b initiates the formation of the terminal C5b-9 complement complex (TCC), called the membrane attack complex (MAC), which, when incorporated into the membranes of pathogens or cells, induces their lysis, or, when incorporated into cells in sublethal doses, induces their activation. Several surface-expressed complement inhibitors that limit the activation of this system to the site of infection and protect host cells from complement attack and lysis tightly regulate the activation of complement [8,73].

instances, complement deficiencies are not associated with an increased frequency of bleeding, and deficiencies of coagulation factors (e.g. hemophilias) do not impair the innate immune response, a recent study has shown that mice deficient in complement component 6 demonstrate prolonged tail bleeding times [9]. Here, we review (i) the structural and physiochemical similarities between the complement and coagulation cascades, (ii) the mechanisms by which complement activation increases blood clotting properties, (iii) 'new pathways' of complement activation through coagulation factors and (iv) the significance of complement-coagulation interactions for the pathogenesis of various clinical conditions.

Complement and coagulation cascades – descendants of a common ancestor

The complement and coagulation systems are organized into proteolytic cascades composed of serine proteases of the chymotrypsin family (Figure 1). The elements of these cascades share several common structural characteristics, including a highly conserved catalytic site composed of Ser, His and Asp [10]. The common principle underlying the organization of these systems is that proteases exist as inactive zymogens and are subsequently activated by upstream, active proteases. The initial activation might occur as a result of contact with a non-enzymatic ligand or cleavage by another protease.

An interesting explanation for the structural and functional similarities between the complement and clotting systems has emerged recently from studies characterizing the serine protease cascade that controls dorsal-ventral patterning in *Drosophila* embryogenesis. These studies

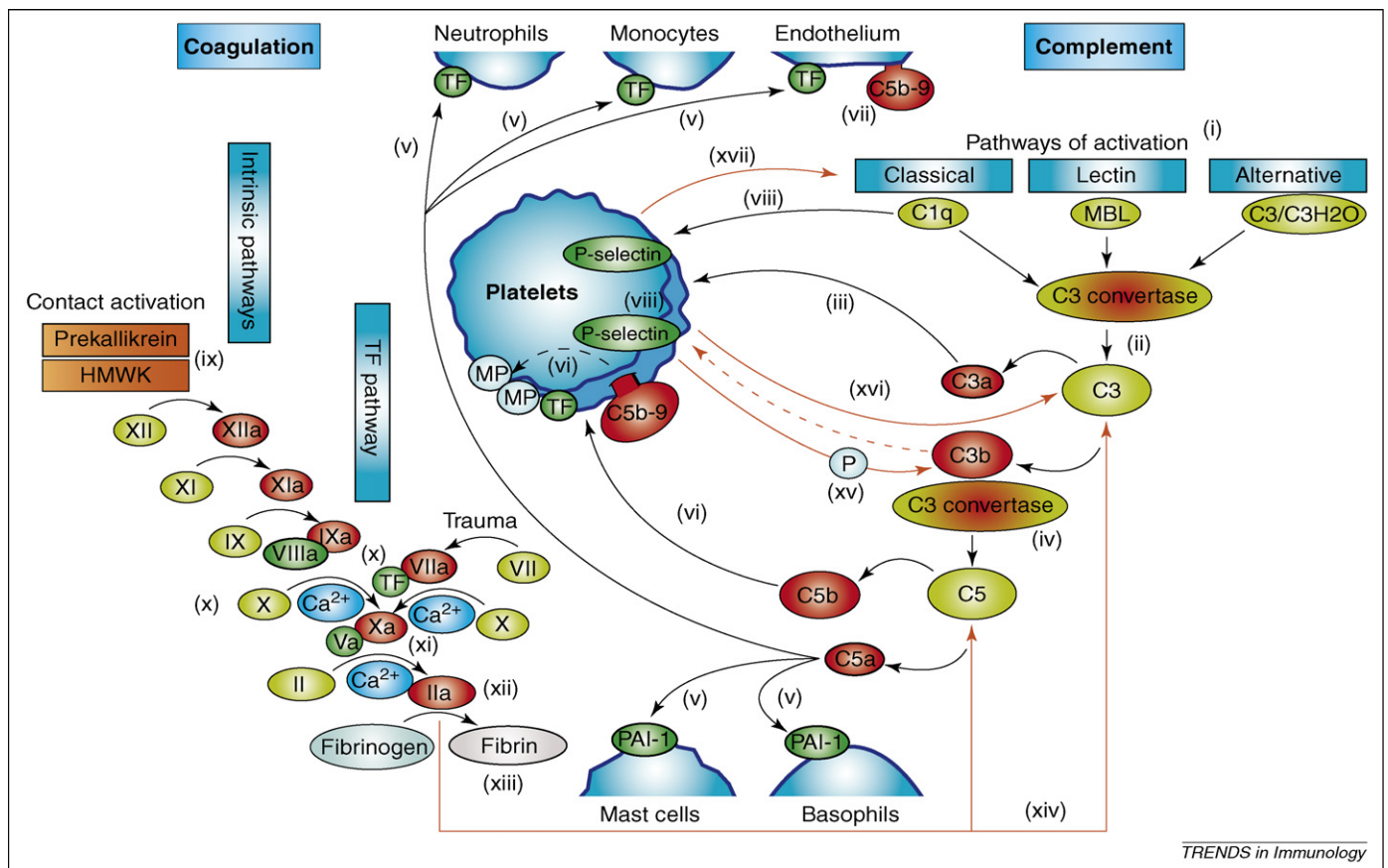
Box 2. Pathways of coagulation

The coagulation system is a component of the homeostatic process and a major contributor to thrombosis (the pathological formation of thrombi within the blood vessels). The coagulation sequence comprises a series of transformations of proenzymes to activated enzymes, culminating in the formation of thrombin (IIa), which converts soluble fibrinogen into insoluble fibrin. With a few exceptions, these enzymatic reactions occur on the phospholipid surface of activated endothelial cells and platelets in the presence of calcium ions. The process of coagulation is activated through the contact (intrinsic) and TF (extrinsic) pathways, both of which converge at the point of activation of factor X. The intrinsic pathway is initiated *in vitro* by contact activation of factor XII, closely linked to the kinin-kallikrein system. The extrinsic pathway, responsible for a rapid and efficient *in vivo* coagulation, is triggered by TF, a cellular lipoprotein released from damaged cells or expressed on the surface of activated monocytes, endothelial cells, and other non-vascular cells. TF converts factor VII to factor VIIa. The process of coagulation is controlled and restricted to the site of vascular injury by natural anticoagulants. These inhibitors of coagulation belong to one of three categories: antithrombins, the protein C and protein S group, or the TF pathway inhibitor system. Dissolution of blood clots (fibrinolysis) is regulated by the plasminogen-plasmin system. This system, activated by PAs, breaks down fibrin and controls fibrin polymerization. PAI-1, produced by various cells including the endothelium, mast cells and basophils, inhibits the activation of this system [74]. Members of the coagulation cascade cooperate closely with platelets, which have a central role in hemostasis. Activated platelets provide negatively charged phospholipid surfaces, where the process of coagulation can occur, and release MPs bearing TF and various mediators, contributing to hemostasis and thrombosis [75].

have made possible a comparison between this system and the complement and clotting cascades in vertebrates and invertebrates [11,12]. The reconstruction of a probable chronology of enzyme evolution using discrete markers of serine protease evolution has led to the hypothesis that the complement and clotting systems originated from a common ancestral developmental-immune cascade existing before the divergence of protostomes and deuterostomes [10]. Among the currently known serine protease cascades, the *Drosophila* dorsal-ventral cascade seems to be the most similar to this ancient ancestor. The extensive similarities among the serine protease cascades from different species suggest that they were built from similar macromolecular units by the continual addition of new enzymes along the pathway [10]. In the light of these findings, the functional linkages between development, immunity and hemostasis observed in vertebrates become clearer. The common origin of the components of proteolytic cascades and, subsequently, the network of their reciprocal interactions explain, at least partially, several new functions of complement and coagulation that have emerged from recent investigations and extend far beyond immunity and hemostasis (Box 3).

Procoagulant properties of complement

Surgery, trauma and severe infections have long been recognized as conditions that predispose to thrombosis [3]. Thrombotic complications have commonly had a more serious impact on the condition of a patient than has the primary disease. The mechanisms underlying this increased tendency for thrombus formation are, in part, related to the procoagulant properties of the inflammatory mediators produced and released as a response to trauma



TRENDS in Immunology

Figure 1. Complement-coagulation reciprocal interactions. Complement and coagulation cascades are composed of serine proteases that are activated through partial cleavage by an upstream enzyme. Zymogens are marked in light green, and active components are shown in red. Complement is activated through the classical, lectin or alternative pathways (i) that converge at the central molecule of the complement system, C3 (ii). The C3 convertases, generated through various pathways, cleave C3 to C3a and C3b (ii). C3a anaphylatoxin activates platelets, enhancing their aggregation and adhesion (iii). C3b contributes to the formation of C5 convertase, which cleaves C5 to C5a and C5b (iv). In addition to its well-established role in inflammation, C5a enhances blood thrombogenicity, mainly through the upregulation of TF and PAI-1 expression on various cell types (v). C5b contributes to the formation of the TCC (also known as C5b-9), which is incorporated into the cellular membrane of platelets, inducing an alteration in membrane polarization and, thus, increasing the surface area on which clotting can occur (vi). TCC also induces the release of MPs bearing TF on their surface (vi) and affects procoagulant properties of endothelium (vii). C3b binds to P-selectin, the expression of which is induced on platelets by C1q (viii), an initiator of the classical pathway of complement activation. Black arrows illustrate the interactions of complement with coagulation, increasing the propensity of blood to clot. Coagulation launched through the contact (intrinsic) pathway begins with contact activation of HMWK, prekallikrein and factor XII (ix). TF expressed on various cells or released from injured cells initiates the physiologically more important TF (extrinsic) pathway (x). Both pathways merge at the level of factor X (xi), which, following activation, converts prothrombin (II) to thrombin (IIa) (xii). The final step of the coagulation process, catalyzed by thrombin, requires partial cleavage of soluble fibrinogen and polymerization to insoluble fibrin (xiii). Thrombin cleaves C3 to C3a and C3b, and C5 to C5a and C5b, thus amplifying the activation of complement (xiv). Platelets, the central cells in hemostasis, also contribute to the amplification of complement through the phosphorylation of C3b (xv), which prolongs the life span of this molecule. Activated platelets are also involved in C3 cleavage (xvi) and initiation of the classical pathway of complement activation (xvii). The amplification of complement activation exerted through the components of the coagulation system is shown as red arrows. Abbreviation: P, P-selectin.

induced by various factors, including infection [2]. In humans with glomerulonephritis or vasculitis, the presence of the products of complement activation, such as C3b, is commonly associated with intravascular coagulation (fibrin deposition) [13].

Complement contributes significantly to thrombosis by directly enhancing blood clotting properties and by augmenting the inflammatory response, which, in turn, potentiates coagulation [2]. The direct procoagulant activities of complement (Figure 1) include its ability to (i) modify phospholipid membranes of cells, which is required for the initiation of the tissue factor (TF) coagulation pathway, (ii) to activate platelets, (iii) to increase TF expression in various cell types [2], and (iv) to modify the activity of mast cells [14] and basophils [15]. The liquidity of blood, a specific status quo existing in non-disturbed systems that is designed to prevent thrombosis, depends on the balance between pro- and anticoagulatory regulators. Therefore, factors influencing the clotting performance of blood,

including complement, could also interfere with anticoagulant regulatory mechanisms.

Complement-mediated modification of cellular surfaces and platelet activation

Coagulation reactions occur on negatively charged phospholipid surfaces, where phosphatidylserine has a key role [16]. This property of clotting reactions ensures that the process is limited to the site of injury at which the formation of a clot is needed. Under steady-state conditions, the outer leaflets of cell membranes do not contain negatively charged phospholipids. However, stimuli that activate coagulation rapidly modify the composition of cellular phospholipid layers, providing a large surface for clot formation. The modification of cellular membranes is an essential event in platelet activation, which is required for primary clot formation [16]. Incorporation of the complement C5b-9 complex [also known as the membrane attack complex (MAC) and the terminal complement complex

Box 3. 'Novel' complement and coagulation cascade functions

Clotting factors participate in immunity, cell growth and embryogenesis. Thrombin is expressed in the liver and also in developing and adult brain [76]. Through its enzymatic properties, it activates protease-activated receptors (PARs) linked to G-protein signal transduction pathways [77], promoting the survival or apoptosis of glial cells and neurons [78,79], the survival of myoblasts [80] and neutrophil chemotaxis [81]. Thrombin also functions nonproteolytically as a chemoattractant for monocytes [82]. Thrombomodulin inhibits thrombin-induced neuronal death [83]. Prothrombin promotes the migration of cells through the extracellular matrix, which is an essential activity for embryonic development and tumor metastasis [84]. Factor Xa functions as a growth factor, stimulating the proliferation of vascular smooth muscle cells [85]. As with the clotting cascade, several 'unconventional' activities have recently been demonstrated for the complement system. Complement proteins contribute to developmental processes such as bone and skeletal development, and they are also involved in the regulation of proliferative and survival pathways. Several complement proteins, including complement regulatory molecules, are involved in mammalian reproduction [36]. In addition, recent studies have shown a regulatory role for the anaphylatoxins C3a and C5a in the priming phase of liver regeneration [37].

(TCC) into the cell membrane activates platelets and results in the exposure of procoagulant lipids [17], the release of microparticles (MPs) providing an extra surface for the conversion of prothrombin to thrombin through prothrombinase (VaXa) [18] and granule secretion from the cytoplasm of platelets [19]. Binding of C1q to its receptor on the surface of platelets induces the expression of integrins and P-selectin, thereby enhancing the procoagulant activity of platelets [20]. The anaphylatoxin C3a induces platelet activation and aggregation [21] (Figure 1).

Induction of TF expression

TF, which initiates the extrinsic pathway of coagulation, is a ubiquitous lipoprotein expressed in high amounts in various tissues, including brain, lung, kidney and placenta. Vascular TF is present in the adventitia, deeply hidden from circulating blood [22]. In this way, coagulation factors can encounter TF only when the integrity of blood vessels is compromised. The other source of TF is the circulating blood leukocytes that upregulate TF when activated by inflammatory mediators [23]. The activation of complement, specifically of C5 as part of an inflammatory response, can cause an increase in the expression of functionally active TF in leukocytes [24] (Figure 1). In addition, C5a [25] and the cytolytically inactive form of the TCC [26] can induce TF expression on human endothelial cells (Figure 1). Although neutrophils carry TF on their membranes, it is unclear whether they themselves can produce TF. However, recent work has shown that complement activation induced by antiphospholipid antibodies and downstream signaling through C5a receptors leads to the induction of TF in these cells [27] (Figure 1).

Role of complement in the activation of the endothelium

Several studies have provided evidence that, in addition to upregulating TF expression, complement effectors contribute to other changes in the endothelium that augment the clotting properties of blood. C5a, in conjunction with

antibodies, induces the shedding of heparan sulfate from the surface of endothelial cells [28]. In normal blood vessels, the heparan sulfate proteoglycan present on the endothelial surface maintains the anticoagulant environment through the localized activation of antithrombin III, a potent inhibitor of thrombin generation [29]. Therefore, this complement activity contributes to an increased propensity for clotting and intravascular coagulation. It has also been suggested that these alterations in the endothelium have an important role in the pathogenesis of hyperacute xenograft rejection and other diseases involving antibody-induced endothelial injury [28]. The upregulation of several adhesion molecules induced by the TCC and C1q can facilitate platelet adhesion to activated endothelial cells [30,26]. In addition, the TCC induces vesiculation of the endothelial cell plasma membrane and exposes catalytic surfaces for the assembly of the prothrombinase enzyme complex on the endothelial cell surface [31]. Another effect of the interaction between the TCC and endothelial cells is an increased vascular permeability of the endothelium monolayer, which can lead to thrombosis following exposure of the subendothelial matrix to the blood [32].

Mast cell switching from a profibrinolytic to a prothrombic phenotype

Mast cells, strategically located in the vicinity of blood vessels and nerves, are among the first responders to the stimuli that initiate inflammation. They also contribute to hemostasis through the expression of tissue type plasminogen activator (t-PA) and heparin production, thereby preventing uncontrolled local activation of the coagulation system. Treating mast cells *in vitro* with C5a causes an upregulation in PA inhibitor 1 (PAI-1) to the extent that the resulting expression of this inhibitor is higher than that of t-PA [14]. This reverse phenotype with regard to t-PA and PAI-1 expression abolishes the fibrinolytic activity of mast cells, leading to a shift in the balance between pro- and anticoagulant factors, in favor of procoagulant factors. C5a also stimulates the production of PAI-1 in basophils [15] (Figure 1).

Inhibition of anticoagulant mechanisms by complement

Complement also augments the thrombogenic properties of blood by inhibiting anticoagulation mechanisms. C4b-binding protein (C4BP), an important cofactor of the enzymatic degradation of C4b, can form a complex with Protein S (PS). PS functions as a cofactor during the degradation of coagulation factors Va and VIIIa by activated protein C (APC), and has a high binding affinity for the negatively charged phospholipids of cell membranes. Formation of the PS-C4BP complex results in a loss of PS cofactor activity, thereby decreasing its anticoagulant effects [33]. Although C4BP behaves as an acute phase protein, and its levels in the blood can increase by up to 400% during an inflammatory reaction, this increase is restricted to the C4BP α^+ form, which does not bind to PS [34]. Therefore, the blood levels of the active free form of PS remain stable even during an acute phase response. The physiological role of PS-C4BP complexes, in particular, highlights the interactions between complement and coagulation. Binding of PS-C4BP

complexes to negatively charged phospholipids, mediated through PS, localizes this complement inhibitor to the sites at which coagulation is initiated. In addition, PS mediates the binding of PS–C4BP complexes to apoptotic cells, most probably through an interaction with negatively charged phosphatidylserine. The deposition of C4BP on the surface of apoptotic cells prevents the activation of the complement cascade beyond C3; therefore, deposited C4BP does not inhibit the early phases of activation that have a role in the uptake of apoptotic cells by phagocytes. This partial inhibition prevents the augmentation of the inflammatory response by apoptotic cells, without interfering with the activation of the mechanisms necessary for the effective elimination of apoptotic cells [33,35].

Indirect procoagulant properties of complement

Occupying a strategic position in the first line of defense in innate immunity, complement exhibits a wide network of interactions with various inflammatory mediators [36]. For example, the complement anaphylatoxins C3a and C5a, generated immediately after the activation of the innate immune response, contribute to the regulation of the cytokine response. Several interactions between anaphylatoxins and the cytokine network have been postulated, including an influence on the production and secretion of tumor necrosis factor (TNF)- α and interleukin (IL)-6 [37]. In turn, TNF- α is a potent enhancer of TF expression on monocytes, and IL-6 increases the production and thrombogenicity of platelets. Inflammatory cytokines also decrease the levels of several anticoagulants, including thrombomodulin, the endothelial cell protein C receptor and PS [38].

Beyond the classical, alternative and lectin pathways of complement activation

During the past few decades, several groups have reported that complement activation is triggered by the activation of the coagulation or contact systems. Early observations have indicated that factors such as thrombin [39] (shown as factor IIa in Figure 1), plasmin [40,41], kallikrein [42] and factor XIIa (also termed Hageman factor) [43] cleave complement components or their fragments *in vitro*. Although these proteolytic cleavage reactions occur at high concentrations of the activated coagulation factors, in conditions associated with a strong inflammatory reaction, such as autoimmune diseases or severe infections, complement–coagulation interactions might be relevant. Recently, the activation of C5 by thrombin [44] (Figure 1) was demonstrated in C3 knockout mice, in which C5 convertases cannot be formed. Human C5 incubated with thrombin was also cleaved to yield biologically active C5a, indicating that C5a generation might occur in the absence of an intact complement system.

Several interactions involving blood cells also contribute to the crosstalk between complement and coagulation. It is well established that complement is activated during the clotting of blood. Significantly higher levels of complement activation fragments are found in human serum than in EDTA-, heparin- or citrate-treated blood [45]. Kalowski *et al.* reported that thrombin and thromboplastin injected into rabbits induce complement activation [46]. Previous induction of thrombocytopenia

attenuates this activation of complement, suggesting that platelets are involved in this phenomenon. This possibility was later confirmed by studies in which platelets in platelet-rich plasma or in whole blood were activated by thrombin receptor-activating peptide (TRAP) [47]. Activated platelets in suspension or immobilized on microtiter plates also seem to activate complement through the classical pathway [48] (Figure 1).

Platelets contain high concentrations of ATP and Ca²⁺ in their dense granules, and when activated, they release these molecules along with Ser/Thr kinases [49]. Both ATP and Ca²⁺ contribute to the extracellular phosphorylation of plasma proteins, including C3, fibrinogen, vitronectin and coagulation factor XI [50]. The phosphorylation of Thr1009 of C3d influences C3 function by increasing the propensity of C3b to bind to activated surfaces. In addition, this phosphorylation attenuates the inactivation of C3b by inhibiting cleavage reactions that are mediated by factor I [49,51]. Overall, C3 phosphorylation prolongs the period during which C3b is active, thereby amplifying complement activation (Figure 1).

Clinical implications of complement–coagulation interactions

Systemic inflammatory response syndrome (SIRS)

Overwhelming systemic activation of inflammation (SIRS, as defined by the consensus conference of the American College of Chest Physicians and Society of Critical Care Medicine in 1991) [52] is central to the pathogenesis of multiorgan trauma and sepsis, which are the leading causes of death in intensive care units. The complement and coagulation cascades are both activated during the course of SIRS: C5a generated in high quantities upregulates TF expression on monocytes, thereby enhancing blood thrombogenicity [7]. Anti-C5a antibody treatment of rats subjected to cecal ligation and puncture (an *in vivo* model of sepsis) remarkably ameliorates the alterations induced by this experimental procedure in the coagulation and fibrinolytic systems, suggesting that complement effectors contribute to the pathogenesis of the coagulation and fibrinolysis disturbances observed in sepsis [53]. The reciprocal interactions occurring between coagulation and complement in SIRS and sepsis might contribute significantly to the amplification of complement activation and blood thrombogenicity that lead to exacerbation of the disease and to fatal complications such as disseminated intravascular coagulation.

Anti-phospholipid syndrome (APS)

APS is a thrombotic disorder associated with the presence of autoantibodies against membrane phospholipids and lipoproteins. Venous and arterial thromboembolism (the formation of thrombi, with subsequent detachment and the blockage of the vessel lumen distal to the site of thrombus formation) and pregnancy loss are the major clinical characteristics of this disorder. APS occurs as a primary autoimmune disease or as a feature of SLE (so-called secondary APS). Complement activation induced by anti-phospholipid autoantibodies contributes to thrombosis in APS, as demonstrated by several *in vivo* studies. Mice deficient in C3 or C5 are less susceptible to

anti-phospholipid antibody-induced thrombosis and endothelial cell activation. In addition, inhibiting C5 activation with an anti-C5 monoclonal antibody prevents the thrombocytopenia that is induced by anti-phospholipid antibodies. Furthermore, mice deficient in C3 or treated with inhibitors of complement activation are protected from fetal loss [54,55].

Systemic lupus erythematosus (SLE)

Thrombosis is recognized as one of the major causes of morbidity and mortality in SLE. Various manifestations of SLE-associated thrombosis include the formation of deep venous thrombi, pulmonary embolism, arterial thrombosis and stroke [6]. Thrombosis-related complications of SLE are caused by antiphospholipid antibodies, which induce alterations in both the complement and coagulation systems [56,57].

Complement regulatory protein dysfunction

In health, the activation of complement is effectively controlled by the coordinated action of soluble and membrane-associated regulatory proteins. Soluble complement regulators such as C1 inhibitor, serum carboxypeptidase N (anaphylotoxin inhibitor), C4BP, factors H and I, clusterin and S-protein (also termed vitronectin) limit the activation of complement at multiple stages of the complement cascade. These regulators are present in both the plasma and body fluids. In addition, host cells are protected against attack by homologous complement by a variety of surface proteins, such as complement receptor 1 (CR1, also known as CD35), membrane cofactor protein (MCP, also known as CD46) and the glycosylphosphatidylinositol (GPI)-anchored proteins decay-accelerating factor (DAF, also known as CD55) and CD59 [8]. However, alterations in the expression of these complement inhibitors can compromise these regulatory mechanisms, leading to excessive complement activation and tissue destruction. Atypical hemolytic-uremic syndrome (aHUS), paroxysmal nocturnal hemoglobinuria (PNH) and hereditary angioedema (HAE) are clinical examples of alterations in complement regulatory protein genes that lead to impaired complement regulatory function and are associated with disturbances in other proteolytic cascades in the plasma.

Atypical hemolytic uremic syndrome (aHUS)

aHUS is a thrombotic microangiopathy characterized by hemolytic anemia, thrombocytopenia and acute renal failure. Several studies have provided evidence that aHUS is associated with mutations in the factor H gene, resulting in an impaired regulation of C3 convertase activity [58,59]. The presence of anti-factor H antibodies has also been associated with aHUS [60]. In addition, mutant forms of factor H have been found to exhibit reduced binding to C3b and C3d, heparin and endothelial cells. These altered binding properties of factor H probably contribute to the progressive damage to endothelial cells and the microvasculature that occur in factor H-associated genetic HUS [61]. Recently, it has been reported that symptoms of HUS are also present in patients carrying mutations in genes encoding other complement regulatory proteins, such as factor I and CD46 [62]. In addition, a subgroup

of aHUS patients with mutations in the gene encoding factor B has been identified. The genetic abnormality in these patients is associated with persistent activation of the alternative complement pathway, further substantiating its importance in the pathogenesis of aHUS [63].

Paroxysmal nocturnal hemoglobinuria (PNH)

PNH is an acquired hematological disease caused by a clonal somatic mutation in the gene encoding PIG-A, which is the GPI anchor. This mutation is associated with a decreased expression of GPI-anchored proteins, including the complement regulators DAF and CD59 [64]. As a result of this decreased expression, the red and white blood cells of PNH patients are highly susceptible to complement-mediated lysis. Thromboembolism is also a frequent complication of this disease, contributing significantly to the increased morbidity and mortality rates in PNH patients. Thrombosis in these patients is associated with elevated levels of TF derived from complement-damaged leukocytes [65]. Treating PNH patients with a humanized anti-C5 antibody markedly reduces intravascular hemolysis and decreased the severity of their symptoms [66].

Hereditary angioedema (HAE)

Hereditary angioedema (HAE) is characterized clinically by the recurrent development of edema in various parts of the body as a result of mutations in the genes encoding C1 inhibitor (C1-INH) or factor XII. Although the pathophysiology of HAE is complex and still the subject of debate, the results of several studies support the hypothesis that the transient and reversible increase in vascular permeability that leads to localized edema is induced by bradykinin, which is formed as a consequence of the activation of the prekallikrein–kallikrein–HMWK (high molecular weight kininogen)–bradykinin system. The activation of this contact system is partially controlled by C1-INH. Therefore, the reduced level (HAE type I) or impaired function (HAE type II) of C1-INH in HAE patients leads to the hyperactivation of C1-INH and the subsequent formation of bradykinin, which binds to the bradykinin receptor 2 [67]. Similarly, mutations in factor XII genes in female carriers lead to an enhancement of factor XII enzymatic activity, which results in an increased production of kinins [68]. Thus, genetic alterations in genes encoding complement and coagulation proteins initiate the common pathophysiologic pathway that connects complement and coagulation with another proteolytic cascade, the contact system. Therefore, HAE is an excellent illustration of the various points of intersection that exist among the plasma proteolytic cascades.

Bioincompatibility

Under physiologic conditions, the surface that is in contact with the blood is restricted to the intact endothelial cell lining of the blood vessels. Activation of complement and clotting are triggered by any disruption of this surface or by the introduction of foreign materials, non-blood cells or microorganisms into the circulation. Bioincompatibility reactions can occur when the biomaterials incorporated in whole blood implants, extracorporeal devices or drug delivery systems come in direct contact with blood and activate both the complement and coagulation cascade

systems. This initial activation results in the subsequent activation of platelets, granulocytes and monocytes, as reflected in thrombotic, anaphylactoid and inflammatory events [69]. Our failure to produce a completely biocompatible material could, in part, be related to our lack of a deeper understanding of the interactions occurring between the proteolytic cascades in the blood when they are activated by biomaterials [69].

The proteolytic cascade systems are activated on biomaterial surfaces by adsorption, a process that is followed by conformational changes in adsorbed plasma proteins. Binding of factor XII to a biomaterial surface triggers contact activation. This molecule is then autoactivated to generate factor XIIa, which cleaves fluid-phase factor XII to yield β -factor XIIa. β -Factor XIIa then activates factor XI and HMWK. Activation of factor XI triggers the coagulation system and leads to the generation of thrombin [69]. An interesting example of crosstalk between the contact system and complement is the activation of the classical pathway reported by Ghebrehwet and coworkers [43], who have described the activation of C1r by β -factor XIIa. In addition, the importance of examining whole blood models under conditions that enable crosstalk between the various biological systems has recently been emphasized [70].

Like the reactions occurring in response to biomaterials, simultaneous activation of complement and coagulation also occurs during transplantation and cell therapies in which cells of non-blood origin are infused into the circulation. A clinical example is the instant blood-mediated inflammatory reaction (IBMIR) that occurs in clinical islet transplantation and other cell transplantation procedures, in which both cascade systems participate [71]. In the IBMIR, complement activation is triggered by the coagulation system, which is activated by TF expressed by the transplanted endocrine cells. Complement activation is attenuated by thrombin inhibitors such as melagatran, despite the observation that melagatran alone does not affect complement [72]. The direct role of complement activation in this process still requires clarification.

Concluding remarks

The activation of the complement system is tightly connected with hemostasis. Multiple regulatory loops linking both systems are simultaneously activated to synchronize an effective response by the host to threats such as infection or bleeding. Most often, this cooperative and clearly beneficial effort assures the elimination of pathogens and prevents life-threatening bleeding. However, when some of the regulatory mechanisms of complement activation or hemostasis fail, complement and hemostatic mechanisms become 'partners in crime', significantly contributing to various pathologies for which only complex therapies targeting multiple molecules can be really effective.

The interaction between complement and coagulation is yet another example of the crosstalk that can occur between various biological systems. The discoveries of modern biology are constantly revealing new links between biological phenomena that were previously thought to belong to unconnected categories. Although these interconnections

make it difficult to precisely define, separate and classify biological processes, this complexity should not discourage scientists as they seek to solve biological 'enigmas'. Instead, researchers need to have a deep awareness of the existence of reciprocal and complex interactions among various reactions that occur *in vivo* as they interpret and seek to understand better the results of their experiments.

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References

- Nathan, C. (2002) Points of control in inflammation. *Nature* 420, 846–852
- Esmon, C.T. (2004) The impact of the inflammatory response on coagulation. *Thromb. Res.* 114, 321–327
- Keel, M. and Trentz, O. (2005) Pathophysiology of polytrauma. *Injury* 36, 691–709
- Sun, H. (2006) The interaction between pathogens and the host coagulation system. *Physiology (Bethesda)* 21, 281–288
- Schroeder, B. *et al.* (1999) Species specificity of plasminogen activation and acquisition of surface-associated proteolytic activity by group C streptococci grown in plasma. *Infect. Immun.* 67, 6487–6495
- Ruiz-Irastorza, G. *et al.* (2001) Systemic lupus erythematosus. *Lancet* 357, 1027–1032
- Guo, R.F. and Ward, P.A. (2005) Role of C5a in inflammatory responses. *Annu. Rev. Immunol.* 23, 821–852
- Walport, M.J. (2001) Complement. First of two parts. *New Engl. J. Med.* 344, 1058–1066
- Bhole, D. and Stahl, G.L. (2004) Molecular basis for complement component 6 (C6) deficiency in rats and mice. *Immunobiology* 209, 559–568
- Krem, M.M. and Di Cera, E. (2002) Evolution of enzyme cascades from embryonic development to blood coagulation. *Trends Biochem. Sci.* 27, 67–74
- Dissing, M. *et al.* (2001) Autoproteolysis and feedback in a protease cascade directing *Drosophila* dorsal-ventral cell fate. *EMBO J.* 20, 2387–2393
- LeMosy, E.K. *et al.* (2001) Activation of a protease cascade involved in patterning the *Drosophila* embryo. *Proc. Natl. Acad. Sci. U. S. A.* 98, 5055–5060
- Nangaku, M. and Couser, W.G. (2005) Mechanisms of immune-deposit formation and the mediation of immune renal injury. *Clin. Exp. Nephrol.* 9, 183–191
- Wojta, J. *et al.* (2003) New aspects in thrombotic research: complement induced switch in mast cells from a profibrinolytic to a prothrombotic phenotype. *Pathophysiol. Haemost. Thromb.* 33, 438–441
- Wojta, J. *et al.* (2002) C5a stimulates production of plasminogen activator inhibitor-1 in human mast cells and basophils. *Blood* 100, 517–523
- Zwaal, R.F. *et al.* (1989) Loss of membrane phospholipid asymmetry during activation of blood platelets and sickled red cells; mechanisms and physiological significance. *Mol. Cell. Biochem.* 91, 23–31
- Sims, P.J. and Wiedmer, T. (1991) The response of human platelets to activated components of the complement system. *Immunol. Today* 12, 338–342
- Sims, P.J. *et al.* (1988) Complement proteins C5b-9 cause release of membrane vesicles from the platelet surface that are enriched in the membrane receptor for coagulation factor Va and express prothrombinase activity. *J. Biol. Chem.* 263, 18205–18212
- Ando, B. *et al.* (1988) Complement proteins C5b-9 initiate secretion of platelet storage granules without increased binding of fibrinogen or von Willebrand factor to newly expressed cell surface GPIIb-IIIa. *J. Biol. Chem.* 263, 11907–11914

- 20 Peerschke, E.I.B. *et al.* (1993) Platelet activation by C1q results in the induction of alpha IIb/beta 3 integrins (GPIIb-IIIa) and the expression of P-selectin and procoagulant activity. *J. Exp. Med.* 178, 579–587
- 21 Polley, M.J. and Nachman, R.L. (1983) Human platelet activation by C3a and C3a des-arg. *J. Exp. Med.* 158, 603–615
- 22 Drake, T.A. *et al.* (1989) Selective cellular expression of tissue factor in human tissues. Implications for disorders of hemostasis and thrombosis. *Am. J. Pathol.* 134, 1087–1097
- 23 Drake, T.A. *et al.* (1989) Functional tissue factor is entirely cell surface expressed on lipopolysaccharide-stimulated human blood monocytes and a constitutively tissue factor-producing neoplastic cell line. *J. Cell Biol.* 109, 389–395
- 24 Muhlfelder, T.W. *et al.* (1979) C5 chemotactic fragment induces leukocyte production of tissue factor activity: a link between complement and coagulation. *J. Clin. Invest.* 63, 147–150
- 25 Ikeda, K. *et al.* (1997) C5a induces tissue factor activity on endothelial cells. *Thromb. Haemost.* 77, 394–398
- 26 Tedesco, F. *et al.* (1997) The cytolytically inactive terminal complement complex activates endothelial cells to express adhesion molecules and tissue factor procoagulant activity. *J. Exp. Med.* 185, 1619–1627
- 27 Ritis, K. *et al.* (2006) A novel C5a receptor–tissue factor cross-talk in neutrophils links innate immunity to coagulation pathways. *J. Immunol.* 177, 4794–4802
- 28 Platt, J.L. *et al.* (1991) The role of C5a and antibody in the release of heparan sulfate from endothelial cells. *Eur. J. Immunol.* 21, 2887–2890
- 29 Marcum, J.A. *et al.* (1986) Cloned bovine aortic endothelial cells synthesize anticoagulant active heparan sulfate proteoglycan. *J. Biol. Chem.* 261, 7507–7517
- 30 Lozada, C. *et al.* (1995) Identification of C1q as the heat-labile serum cofactor required for immune complexes to stimulate endothelial expression of the adhesion molecules E-selectin and intercellular and vascular cell adhesion molecules 1. *Proc. Natl. Acad. Sci. U. S. A.* 92, 8378–8382
- 31 Hamilton, K.K. *et al.* (1990) Complement proteins C5b-9 induce vesiculation of the endothelial plasma membrane and expose catalytic surface for assembly of the prothrombinase enzyme complex. *J. Biol. Chem.* 265, 3809–3814
- 32 Bossi, F. *et al.* (2004) Platelet-activating factor and kinin-dependent vascular leakage as a novel functional activity of the soluble terminal complement complex. *J. Immunol.* 173, 6921–6927
- 33 Rezende, S.M. *et al.* (2004) Coagulation, inflammation, and apoptosis: different roles for protein S and the protein S–C4b binding protein complex. *Blood* 103, 1192–1201
- 34 Garcia de Frutos, P. *et al.* (1994) Differential regulation of α and β chains of C4b-binding protein during acute-phase response resulting in stable plasma levels of free anticoagulant protein S. *Blood* 84, 815–822
- 35 Trouw, L.A. *et al.* (2005) C4b-binding protein binds to necrotic cells and DNA, limiting DNA release and inhibiting complement activation. *J. Exp. Med.* 201, 1937–1948
- 36 Mastellos, D. *et al.* (2005) Novel biological networks modulated by complement. *Clin. Immunol.* 115, 225–235
- 37 Markiewski, M.M. *et al.* (2006) Liver inflammation and regeneration: Two distinct biological phenomena or parallel pathophysiologic processes? *Mol. Immunol.* 43, 45–56
- 38 Shebuski, R.J. and Kilgore, K.S. (2002) Role of inflammatory mediators in thrombogenesis. *J. Pharmacol. Exp. Ther.* 300, 729–735
- 39 Spath, P. and Gabl, F. (1976) Critical role of the conversion of the third complement component C3 (β 1C/ β 1A) for its immunochemical quantitation. *Clin. Chim. Acta* 73, 171–175
- 40 Goldberger, G. *et al.* (1981) NH₂-terminal structure and cleavage of guinea pig pro-C3 the precursor of the third complement component. *J. Biol. Chem.* 256, 12617–12619
- 41 Lachmann, P.J. *et al.* (1982) Breakdown of C3 after complement activation. Identification of a new fragment, C3g, using monoclonal antibodies. *J. Exp. Med.* 156, 205–216
- 42 Thoman, M.L. *et al.* (1984) C3d-K, a kallikrein cleavage fragment of iC3b is a potent inhibitor of cellular proliferation. *J. Immunol.* 133, 2629–2633
- 43 Ghebrehwet, B. *et al.* (1983) Mechanisms of activation of the classical pathway of complement by Hageman factor fragment. *J. Clin. Invest.* 71, 1450–1456
- 44 Huber-Lang, M. *et al.* (2006) Generation of C5a in the absence of C3: a new complement activation pathway. *Nat. Med.* 12, 682–687
- 45 Mollnes, T.E. *et al.* (1988) Effect of time, temperature and anticoagulants on *in vitro* complement activation: consequences for collection and preservation of samples to be examined for complement activation. *Clin. Exp. Immunol.* 73, 484–488
- 46 Kalowski, S. *et al.* (1975) Effects of intravascular clotting on the activation of the complement system: The role of the platelet. *Am. J. Pathol.* 78, 525–536
- 47 Del Conde, I. *et al.* (2005) Platelet activation leads to activation and propagation of the complement system. *J. Exp. Med.* 201, 871–879
- 48 Peerschke, E.I. *et al.* (2006) Blood platelets activate the classical pathway of human complement. *J. Thromb. Haemost.* 4, 2035–2042
- 49 Ekdahl, K.N. and Nilsson, B. (1995) Phosphorylation of complement component C3 and C3 fragments by a human platelet protein kinase. Inhibition of factor I-mediated cleavage of C3b. *J. Immunol.* 154, 6502–6510
- 50 Ekdahl, K.N. *et al.* (1997) Increased phosphate content in complement component C3, fibrinogen, vitronectin, and other plasma proteins in systemic lupus erythematosus: covariation with platelet activation and possible association with thrombosis. *Arthritis Rheum.* 40, 2178–2186
- 51 Ekdahl, K.N. and Nilsson, B. (1999) Alterations in C3 activation and binding caused by phosphorylation by a casein kinase released from activated human platelets. *J. Immunol.* 162, 7426–7433
- 52 No authors listed (1992) American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit. Care Med.* 20, 864–874
- 53 Laudes, I.J. *et al.* (2002) Anti-c5a ameliorates coagulation/fibrinolytic protein changes in a rat model of sepsis. *Am. J. Pathol.* 160, 1867–1875
- 54 Pierangeli, S.S. *et al.* (2005) Complement activation: a novel pathogenic mechanism in the antiphospholipid syndrome. *Ann. N. Y. Acad. Sci.* 1051, 413–420
- 55 Holvers, V.M. *et al.* (2002) Complement C3 activation is required for antiphospholipid antibody-induced fetal loss. *J. Exp. Med.* 195, 211–220
- 56 Inanc, M. *et al.* (1998) Anti- β 2-glycoprotein I, anti-prothrombin and anticardiolipin antibodies in a longitudinal study of patients with systemic lupus erythematosus and the antiphospholipid syndrome. *Br. J. Rheumatol.* 37, 1089–1094
- 57 Nojima, J. *et al.* (1999) Platelet activation induced by combined effects of anticardiolipin and lupus anticoagulant IgG antibodies in patients with systemic lupus erythematosus – possible association with thrombotic and thrombocytopenic complications. *Thromb. Haemost.* 81, 436–441
- 58 Warwicker, P. *et al.* (1998) Genetic studies into inherited and sporadic hemolytic uremic syndrome. *Kidney Int.* 53, 836–844
- 59 Rougier, N. *et al.* (1998) Human complement factor H deficiency associated with hemolytic uremic syndrome. *J. Am. Soc. Nephrol.* 9, 2318–2326
- 60 Dragon-Durey, M.A. *et al.* (2005) Anti-factor H autoantibodies associated with atypical hemolytic uremic syndrome. *J. Am. Soc. Nephrol.* 16, 555–563
- 61 Manuelian, T. *et al.* (2003) Mutations in factor H reduce binding affinity to C3b and heparin and surface attachment to endothelial cells in hemolytic uremic syndrome. *J. Clin. Invest.* 111, 1181–1190
- 62 Caprioli, J. *et al.* (2006) Genetics of HUS: the impact of MCP, CFH, and IF mutations on clinical presentation, response to treatment, and outcome. *Blood* 108, 1267–1279
- 63 Goicoechea de Jorge, E. *et al.* (2007) Gain-of-function mutations in complement factor B are associated with atypical hemolytic uremic syndrome. *Proc. Natl. Acad. Sci. U. S. A.* 104, 240–245
- 64 Takeda, J. *et al.* (1993) Deficiency of the GPI anchor caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal hemoglobinuria. *Cell* 73, 703–711
- 65 Liebman, H.A. and Feinstein, D.I. (2003) Thrombosis in patients with paroxysmal nocturnal hemoglobinuria is associated with markedly elevated plasma levels of leukocyte-derived tissue factor. *Thromb. Res.* 111, 235–238
- 66 Hillmen, P. *et al.* (2006) The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *New Engl. J. Med.* 355, 1233–1243

- 67 Davis, A.E., III (2005) The pathophysiology of hereditary angioedema. *Clin. Immunol.* 114, 3–9
- 68 Cichon, S. *et al.* (2006) Increased activity of coagulation factor XII (Hageman factor) causes hereditary angioedema type III. *Am. J. Hum. Genet.* 79, 1098–1104
- 69 Gorbet, M.B. and Sefton, M.V. (2004) Biomaterial-associated thrombosis: roles of coagulation factors, complement, platelets and leukocytes. *Biomaterials* 25, 5681–5703
- 70 Mollnes, T.E. *et al.* (2002) Essential role of the C5a receptor in *E. coli*-induced oxidative burst and phagocytosis revealed by a novel lepirudin-based human whole blood model of inflammation. *Blood* 100, 1869–1877
- 71 Moberg, L. *et al.* (2002) Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet* 360, 2039–2045
- 72 Ozmen, L. *et al.* (2002) Inhibition of thrombin abrogates the instant blood-mediated inflammatory reaction triggered by isolated human islets: possible application of the thrombin inhibitor melagatran in clinical islet transplantation. *Diabetes* 51, 1779–1784
- 73 Walport, M.J. (2001) Complement. Second of two parts. *New Engl. J. Med.* 344, 1140–1144
- 74 McVey, J.H. (1999) Tissue factor pathway. *Best Pract. Res. Clin. Haematol.* 12, 361–372
- 75 Stassen, J.M. *et al.* (2004) The hemostatic system. *Curr. Med. Chem.* 11, 2245–2260
- 76 Dihanich, M. *et al.* (1991) Prothrombin mRNA is expressed by cells of the nervous system. *Neuron* 6, 575–581
- 77 Ishihara, H. *et al.* (1997) Protease-activated receptor 3 is a second thrombin receptor in humans. *Nature* 386, 502–506
- 78 Donovan, F.M. *et al.* (1997) Thrombin induces apoptosis in cultured neurons and astrocytes via a pathway requiring tyrosine kinase and RhoA activities. *J. Neurosci.* 17, 5316–5326
- 79 Donovan, F.M. and Cunningham, D.D. (1998) Signaling pathways involved in thrombin-induced cell protection. *J. Biol. Chem.* 273, 12746–12752
- 80 Chinni, C. *et al.* (1999) Thrombin, a survival factor for cultured myoblasts. *J. Biol. Chem.* 274, 9169–9174
- 81 Bizios, R. *et al.* (1986) Thrombin-induced chemotaxis and aggregation of neutrophils. *J. Cell. Physiol.* 128, 485–490
- 82 Bar-Shavit, R. *et al.* (1983) Monocyte chemotaxis: stimulation by specific exosite region in thrombin. *Science* 220, 728–731
- 83 Sarker, K.P. *et al.* (1999) Inhibition of thrombin-induced neuronal cell death by recombinant thrombomodulin and E5510, a synthetic thrombin receptor signaling inhibitor. *Thromb. Haemost.* 82, 1071–1077
- 84 Henrikson, K.P. *et al.* (1999) Role of thrombin receptor in breast cancer invasiveness. *Br. J. Cancer* 79, 401–406
- 85 Kaiser, B. *et al.* (2000) A synthetic inhibitor of factor Xa, DX-9065a, reduces proliferation of vascular smooth muscle cells *in vivo* in rats. *Thromb. Res.* 98, 175–185

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