REVIEW ARTICLES

Overview of Blood Coagulation

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The endothelium is the principal anti-thrombotic mechanism, providing a non wettable surface and generating potent vasodilators (nitric oxide and prostacyclin) and clotting inhibitors [thrombomodulin and tissue factor pathway inhibitor (TFPI)]. When the integrity of the endothelium is breached, vasoconstriction occurs through neural and chemical (endothelin, thromboxane) mechanisms, and platelet adhesion is facilitated (von Willebrand factor). Activation of platelets accompanied by microparticle formation provides a thrombogenic surface for subsequent coagulation reactions. The initial generation of small amounts of thrombin greatly amplifies subsequent clotting factor activation and results in substantial thrombin formation. Thrombin activates an inhibitor of fibrinolysis [thrombin activatable fibrinolysis inhibitor (TAFI)] which prevents the binding of plasminogen to fibrin. Mechanisms to limit clot formation include inhibition of the tissue factor–factor VIIa complex by TFPI, inhibition of activated factors V and VIII by activated protein C, and binding of thrombin by thrombomodulin, heparin cofactor II, and anti-thrombin. Clot dissolution is promoted by plasminogen activators (tissue plasminogen activator and urokinase) and by plasminogen.

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Key words

Coagulation, thrombin, fibrin, fibrinolysis, platelets, endothelium

Initiation of coagulation

When a vessel is injured, it undergoes reflex vasospasm, which is sustained by the release of endothelin, a potent vasoconstrictor. Shear stress at the site of injury, as well as calcium fluxes, induces the release of von Willebrand factor from specialized organelles present in endothelial cells, the Weibel–Palade bodies [1]. The von Willebrand factor promotes platelet adherence to subendothelial connective tissue, mediated by platelet glycoprotein Ib. The spreading platelets express P-selectin, which may slow the rolling of polymorphonuclear leukocytes (PMNs) on the vessel surface and activate them [2]. The PMNs are also a source of tissue factor that becomes adherent to platelets [3]. Once activated, leukocytes can directly activate coagulation factor X to factor Xa [4]. In addition, endothelial cells release chemoattractants for monocytes and macrophages; these cells become activated and express tissue factor on their surfaces.

Adherent platelets become activated and release thromboxane-A_2, which induces vasoconstriction and platelet aggregation. They also release adenosine diphosphate and calcium, both of which reinforce the formation of platelet aggregates [5]. Other proteins released by platelets include the von Willebrand factor; platelet factor 4, which neutralizes heparin and other proteoglycans; and factor V and fibrinogen, which promote coagulation. Lastly, platelets generate microparticles or membrane vesicles, which provide a large thrombogenic surface for the localization of clotting factors.

Clinical relevance

Deficiencies or defects in either von Willebrand factor or its binding site on platelets (glycoprotein Ib) result in von Willebrand disease or Bernard–Soulier syndrome, respectively. These conditions are characterized by impaired platelet adhesion under high shear, resulting in prolonged bleeding from wounds to the skin or mucous membranes. On the other hand, an excess of high molecular weight multimers of the von Willebrand factor occurs in thrombotic thrombocytopenic purpura. The excess is attributed to antibodies directed against the protease that normally cleaves von Willebrand factor [6]. The result is the formation of platelet thrombi throughout the microvasculature. The role of the von Willebrand factor in hemolytic–uremic syndrome remains to be clarified [7].

The administration of drugs such as aspirin impairs platelet release of thromboxane, resulting in a mild bleeding tendency. Phosphodiesterase inhibitors such as dipyridamole raise platelet cyclic AMP levels, rendering the platelet less responsive to agonists. Ticlopidine and clopidogrel inhibit adenosine diphosphate–induced platelet aggregation by blocking the platelet receptor $P2Y_{ADP}$ [8]. More potent inhibition of plate-

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let function occurs with abciximab, which binds to platelet glycoprotein IIb/IIIa and prevents the platelet release reaction. Platelet microparticles are powerful procoagulants and are found in pathologic thrombotic disorders such as heparin-induced thrombocytopenia [9] and the disseminated intravascular coagulation (DIC) associated with malignancies.

Thrombin generation

Thrombin generation is dependent on the formation of three procoagulant enzyme complexes, each of which consists of a vitamin K–dependent serine protease (factor VII, IX, X, or prothrombin) associated with a membrane-bound cofactor (tissue factor, factor V, or factor VIII) assembled on a membrane surface (Fig. 1) [10]. The first such complex consists of factor VII, which becomes bound to tissue factor expressed on the membrane of activated monocytes and macrophages. Binding to tissue factor activates factor VII to factor VIIa [11]. The tissue factor–VIIa complex activates factors IX and X (to IXa and Xa, respectively). The second complex (the prothrombinase complex) consists of factor Xa and its cofactor, factor V, and prothrombin. This complex converts prothrombin to thrombin. Thrombin activates factors V, VIII, and XI, thereby amplifying its own generation. Factor XIa activates additional factor IX. The final complex (the tenase complex) consists of factor IXa and its cofactor, factor VIIIa, and factor X, which is converted to factor Xa. The relatively large amounts of factor Xa so generated lead to the formation of sufficient thrombin to form hemostatically effective fibrin clots.

Clinical relevance

Platelets play a major role in thrombus formation by releasing procoagulants, providing a large surface area for the binding of clotting factors, and protecting activated coagulants from inhibitors. This behavior explains why thrombocytopenia is associated with bleeding, and thrombocytosis with thrombosis. The major components of the procoagulant complexes are vitamin K–dependent serine proteases produced by the liver. Thus, vitamin K deficiency and liver disease are associated with bleeding. Deficiency of vitamin K may be due to poor nutrition; it also occurs with malabsorption syndromes; and it is deliberately induced by warfarin therapy.

The expression of tissue factor occurs with vascular injury, as with the erosion of an atheromatous plaque, or with widespread tissue trauma such as occurs with burns, major surgery, or some obstetrical conditions. Many neoplasms expose membrane-associated tissue factor. Endotoxin is also a powerful inducer of monocyte and macrophage tissue factor. Local exposure of tissue factor promotes thrombus formation at injury sites and on plaques; but, with crush injuries, extensive trauma, metastatic malignancy, and sepsis, the widespread exposure of tissue factor results in DIC [12].

Fibrin formation and inhibition of fibrinolysis

Thrombin cleaves two peptides from the α and β chains of fibrinogen (fibrinopeptides A and B) to form fibrin monomer. Fibrin monomers aggregate and form fibrin polymers. Thrombin activates factor XIII, forming the active A_2 subunit, which then dissociates from the B_2 carrier protein; the A_2 subunit cross-links the fibrin chains. Thrombin activates a fibrinolysis inhibitor (TAFI; Fig. 2) which prevents the binding of plasminogen to fibrin [14]. Activated platelets release another inhibitor of fibrinolysis, plasminogen activator in-

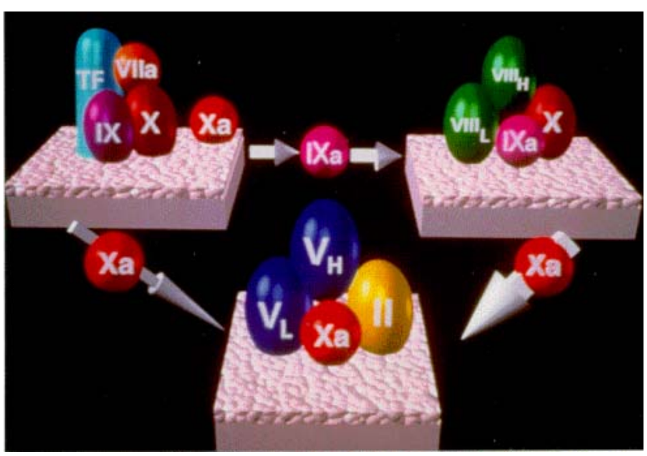

FIGURE 1 Procoagulant coagulation complexes. The first complex generates activated factor IX (IXa) and small amounts of activated factor X (Xa). The tenase complex generates large amounts of Xa, and the prothrombinase complex converts prothrombin (II) to thrombin. TF = tissue factor; H and L (subscripts) = heavy and light chains of factors V and VIII. (Reproduced from [10].)

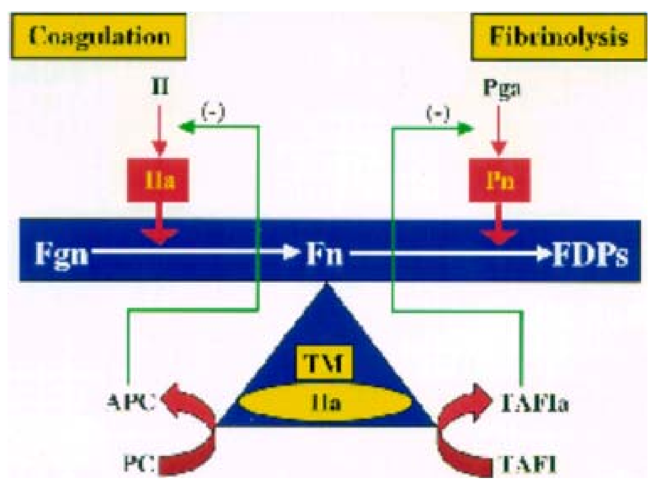

FIGURE 2 Regulation of coagulation and fibrinolysis. The thrombomodulin (TM)–thrombin (IIa) complex catalyzes the formation of activated protein C (PC → APC), which indirectly inhibits thrombin generation from prothrombin (II). It also catalyzes the activation of thrombin-activatable fibrinolysis inhibitor (TAFI → TAFIa), which inhibits plasmin generation from plasminogen activator (Pga → Pn). Thrombin catalyzes the cleavage of fibrinogen (Fgn) to form fibrin (Fn). Plasmin lyses fibrin to form fibrin degradation products (FDPs). (Reproduced with permission from [13].)

hibitor-1 (PAI-1). Other inhibitors of fibrinolysis include plasmin inhibitor (α_1-antiplasmin) and lipoprotein(a), which competes with plasminogen for binding to fibrinogen [15].

Clinical relevance

Patients with hemophilia have delayed thrombin generation and therefore produce less TAFI. In consequence, their fibrinolysis is more active. This situation probably contributes to their bleeding problems—in particular, to the late-onset bleeding that is characteristic of hemophilia. Fibrinolysis is also much more active in patients with liver disease, for two reasons: first, hepatic clearance of tissue plasminogen activator is decreased; and second, synthesis of inhibitors of fibrinolysis, such as plasmin inhibitor and α_2-macroglobulin, is reduced.

Adipocytes synthesize PAI-1, which is elevated in people with obesity [16]. Obesity is associated with an increased risk for venous thrombosis. In addition, high concentrations of PAI-1 are found in metabolic syndrome, which is characterized by insulin resistance and severe atherothrombotic disease [17]. Increased levels of lipoprotein(a) have been associated with a heightened risk for both venous [18] and arterial thrombosis.

Factors limiting coagulation

The endothelium releases nitric oxide and prostacyclin, potent vasodilators and inhibitors of platelet aggregation. The endothelium also provides tissue factor pathway inhibitor (TFPI), which forms a complex with factor Xa and inhibits the tissue factor–VIIa complex [19]. Proteins such as annexin V and β_2-glycoprotein-1 bind to negatively charged phospholipids on activated platelets, preventing attachment of coagulation factors [20]. Heparin-like proteoglycans in the subendothelium activate heparin cofactor II and antithrombin, which inhibit thrombin [21]. Antithrombin also inactivates several clotting factors (IXa, Xa, XIa) and the tissue factor–VIIa complex [22].

Thrombomodulin, a protein expressed by the endothelium, binds thrombin; the resulting complex activates protein C (Fig. 2). Activated protein C (aPC), with protein S as a cofactor, inactivates factor Va. Furthermore, aPC, with factor V as a cofactor, inactivates factor VIIIa [23]. Thus, aPC is a major inhibitor of thrombin generation. About one third of protein S is free in the plasma; the other two thirds are bound to the C4b-binding protein. Bound protein S is incapable of acting as a cofactor for aPC. β_2-Glycoprotein-1 competes with the C4b-binding protein for protein S, increasing the availability of free protein S [24].

Clinical relevance

A breach in the integrity of the endothelium promotes thrombosis. This effect may be readily observed in patients undergoing balloon angioplasty; it is controlled by the administration of inhibitors of platelet function and the insertion of stents to maintain vessel patency. People with the antiphospholipid antibody syndrome may have auto-antibodies directed against β_2-glycoprotein-1 or annexin V, or both; the presence of such auto-antibodies increases the risk of venous or arterial thrombosis and intrauterine fetal death due to placental insufficiency [25].

Deficiencies of antithrombin, protein C, and protein S are associated with hypercoagulability. Inherited disorders of these physiologic anticoagulants are relatively rare, but many patients have acquired deficiencies owing to a variety of diseases. For example, plasma levels are low in liver disease and in DIC. Antithrombin is inactivated and excreted in the urine of patients with nephrotic syndrome [26]. Concentrations of proteins C and S are reduced by warfarin therapy or vitamin K deficiency, and low levels of protein C are encountered in patients with severe sepsis [27]. Other causes of thrombophilia include mutations in the genes for factor V (factor V Leiden) and prothrombin (prothrombin G20210A), which result in a gain of function for these procoagulants [28].

Activation of fibrinolysis

As fibrin forms in the blood, it binds the major activator of the fibrinolytic system, tissue plasminogen activator (t-PA) [29]. In addition, plasminogen also binds to fibrin and is activated to plasmin by t-PA. However, plasmin inhibitor and plasminogen activator inhibitor-1 (PAI-1) also bind to fibrin and inhibit plasminogen activation and plasmin. Plasmin lyses the fibrin clot; plasmin residing in the fibrin meshwork is protected from inactivation by plasmin inhibitor.

Clinical relevance

Activators of plasminogen such as streptokinase and t-PA are widely used to lyse thrombi in patients with major organ ischemia. It is not generally appreciated that the efficacy of these lytic agents depends on plasminogen levels. With repeated administration of the activators, plasminogen is consumed and the thrombolytic activity of the agent may be reduced. Infusions of plasma may restore plasminogen levels and lytic activity. If fibrinolysis is excessive, drugs such as epsilon aminocaproic acid may be given to inhibit fibrinolytic activity. Such agents are very useful in patients with liver disease who have excessive lytic activity owing to failure of the liver to produce adequate amounts of plasmin inhibitor.

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