

The hemostatic system. 1st Part

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Abstract

The hemostatic system is a complex ancestral pathway physiologically dedicated to protect the individual from bleeding. It starts immediately after an endothelial injury. Platelets and blood coagulation act synergically to provide a strength clot able to stop bleeding. In healthy subjects, the hemostatic system is able to work to avoid an excess of fibrin formation and deposition within the blood vessels on the one hand but is ready to stop bleeding on the other. To reach this crucial objective, a fine regulation of its activity is required. In other words, all actions of the hemostatic system are under control to assure a perfect balance to maintain people distant from both Scylla (bleeding) and Charybdis (thrombosis). Fibrinolysis is a complementary defensive system essential to regulate fibrin deposition via its dissolution. It is, in turn, well controlled to avoid bleeding and thrombosis by a fine control of its inducers and inhibitors. The aim of this review is to provide a picture of global haemostasis for helping in understanding this complex topic.

Keywords

Primary hemostasis, platelets, blood coagulation, Vitamin K, fibrin formation, fibrinolysis.

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Introduction

Hemostasis is a complex phenomenon. Blood coagulation and platelets belong to a defensive system developed against bleeding. Blood

coagulation starts immediately after a lesion of the endothelium, coordinated with platelets, which form a hemostatic plug providing a first block of the hemorrhage [1]. Clot is the final product of blood coagulation activation triggered by extravascular compartment rich in Tissue Factor (TF), a molecule able to trigger the coagulative cascade [2]. Fibrinolysis is a further step of the system because its role consists in the digestion of the clot [3]. All these actions are under the control of natural anticoagulants and fibrinolysis inhibitors. This system is always working, such as a car engine idles, since fibrin deposition and dissolution in the endothelium is a continuous phenomenon. It is ready to greatly accelerate its function when either bleeding or a vascular occlusion occurs. In this review, we will describe the hemostatic function of platelets and blood coagulation, followed by that of fibrinolysis.

Primary hemostasis

Primary hemostasis is due to platelets, which are small fragments that come from megakaryocytes, large cells located in the bone marrow [4]. Normal platelet count ranges from 150 to 450 x 10⁹/L, and they live in the circulation for about 10 days [5]. Normally, platelets do not adhere to surfaces or aggregate within the blood vessels. However, if an endothelial damage occurs, they are ready to start a contact with the sub-endothelial matrix leading to adhesion and aggregation [6]. Platelets receptor GPIb-IX-V binds to A1 domain of von Willebrand Factor (vWF), a large multimeric protein released from endothelial cells and megakaryocytes that is present both in plasma and in the sub-endothelial matrix [7]. Another receptor dedicated to platelet adhesion is GPVI, which binds to the collagen of the sub-endothelial matrix at the side of injury [8]. Adhesion of platelets to the endothelial matrix leads to their activation, which consists in a conformational change of α IIb β 3. It is an integrin, that exposes, after clustering on the membrane, binding sites for fibrinogen, vWF, collagen and fibronectin, thus inducing platelet aggregation [9]. Other integrins are involved in this pathway but have a less important role. However, a further activation of platelets for an optimal aggregation and formation of the hemostatic plug is required. This activation is induced by agonists released by platelets themselves, such as Adenosine Diphosphate (ADP), which binds

to receptors P2Y1 and P2Y2, and Thromboxane A2 (TXA2) whose synthesis happens inside the platelets when activated. TXA2 then binds to its receptor on the platelet membrane, further enhancing platelet aggregation [10]. Another agonist is serotonin, which is released from activated platelets contributing to platelet aggregation [11]. Moreover, a further mechanism that enhances platelet aggregation is that of thrombin, the final protease of blood coagulation. Thrombin cleaves two Protease-Activated Receptors (PARs) on platelets, thus starting a cell signaling process that results in platelet granule secretion, integrin activation and a cytoskeleton remodelling, i.e. the Phosphatidylserine (PS) exposure. PS translocation to the outer leaflet, in turn, contributes both to the absorption of blood coagulation factors and to the release of the stored ones, thus facilitating further thrombin formation [12]. PS-exposing platelets also shed extracellular vesicles (microparticles) [13], which have the opportunity to further amplify the hemostatic and coagulative properties of platelets (Fig. 1).

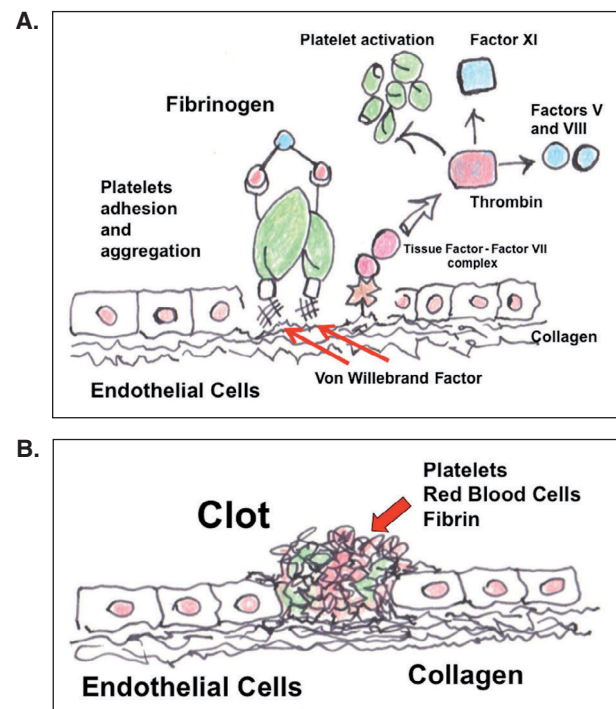


Figure 1. Primary haemostasis (A) and clot formation (B). After endothelium injury, adhesion and aggregation of platelets mediated by von Willebrand Factor and fibrinogen, respectively, occur. A small amount of thrombin is concomitantly produced by Tissue Factor exposed by fibroblast cells of the sub-endothelial space and Factor VII. Thrombin is able to enhance its production by activating Factors XI, V, VIII and platelets. The final outcome is a stable clot that reinforces the first hemostatic platelet plug. Red Blood Cells are involved within the clot.

Blood coagulation

Blood coagulation *in vivo* is triggered by TF, a 47-kDa type 1 transmembrane glycoprotein, abundant in the sub-endothelial space, which forms a complex with Factor VII (FVII) once the endothelial barrier is interrupted [14]. A small amount of thrombin is then formed, which in turn amplifies the coagulation cascade activating Factor XI (FXI) [15] on the one hand and platelets on the other. Thrombin is the product of the coagulation cascade activated by the complex TF-FVII that activates Factor X (FX). However, FX is also activated by Factor IX (FIX), which belongs to the waterfall intrinsic activation of blood coagulation involving Factors XII (FXII) and FXI. This enzymatic reaction is greatly accelerated by Factor VIII (FVIII). In other words, a loop named “the Josso’s triangle” is therefore formed [16]. Many years after, a positive feedback has been discovered: the TF-FVIIa and TF-FVIIa-FXa complexes are able also to activate FVIII. This mechanism can, therefore, enhance the active FVIIIa-FIXa (intrinsic Xase) confirming the existence of the Josso’ triangle [17] (**Fig. 2**).

FXa is able to activate prothrombin to thrombin with the acceleration of Factor V (FV) (the prothrombinase complex). Platelet activation is an essential step since it provides a phospholipid surface (phosphatidylserine) on which the adsorption of clotting factors is optimal.

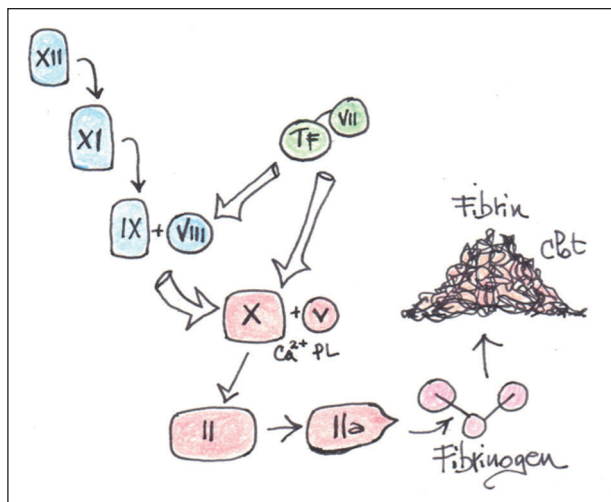


Figure 2. The waterfall enzymatic activation of blood coagulation. A pivotal role is that of the Factor Xa activation by both the complexes Tissue Factor-Factor VII and Factor IX-Factor VIII (the Josso’s triangle). Fibrin is the final product.

TF: Tissue Factor, II: Prothrombin, IIa: Thrombin, PL: Phospholipids.

Thrombin, which is able to induce also the activation of FVIII and FV, further enhances the coagulation burden and it is now ready to activate fibrinogen to fibrin, an insoluble clot for preventing blood loss and inducing wound healing [18].

A negative feedback is provided by a series of natural anticoagulants, which control blood coagulation, avoiding an excess of activity. Antithrombin and the system of Protein C are the main actors that limit the activation of thrombin, thus obtaining a perfect balance between blood coagulation activation and its inhibition. First, thrombin becomes an anticoagulant factor after binding to Thrombomodulin (TM), an endothelium-bound glycoprotein. Once this complex is formed, thrombin loses its capacity to transform fibrinogen into fibrin on the one hand but activates Protein C, a Vitamin K dependent protein, on the other. Activated Protein C binds to Protein S, another Vitamin K dependent protein, forming a new complex, which inactivates FV and FVIII proteolytically, so downregulating thrombin formation [19]. Secondly, antithrombin, a member of the serine protease inhibitors, inactivates multiple coagulation factors, mainly thrombin and FXa, and to a lesser extent FIXa, FXIa, and FXIIa [20] (**Fig. 3**). Third, the TF pathway inhibitor limits the activity of the TF-FVII complex, the main trigger of blood coagulation *in vivo* [21].

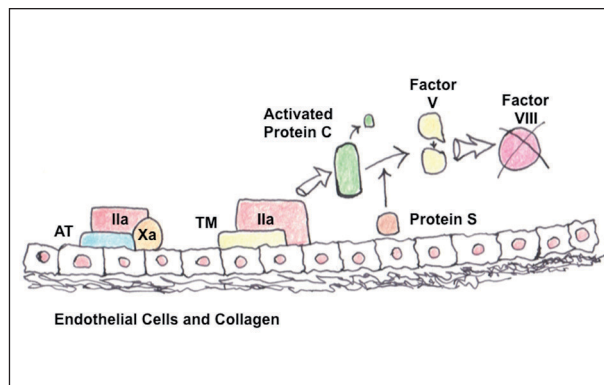


Figure 3. The main negative feedbacks of blood coagulation. Antithrombin inactivates mainly thrombin and Factor Xa, and to a lesser extent Factor IXa, Factor XIa, and Factor XIIIa. Thrombin becomes an anticoagulant factor after binding to Thrombomodulin (TM); once this complex is formed, thrombin loses its capacity to transform fibrinogen into fibrin on the one hand but activates Protein C, on the other. Activated Protein C binds to Protein S, forming a new complex that inactivates Factor V and Factor VIII proteolytically so downregulating thrombin formation. AT: Antithrombin; IIa: Thrombin; TM: Thrombomodulin.

The role of Vitamin K

Vitamin K has an important role in blood coagulation as an anti-haemorrhagic factor. Vitamin K (*Koagulation*) is a fat-soluble vitamin identified by the Danish biochemist Henrik Dam in 1934 [22] after he demonstrated that chicks fed with a low-fat diet showed a hemorrhagic disease. Dam noticed that this vitamin was present in abundance in pig liver fat but also in plants.

Vitamin K identifies a group of lipophilic and hydrophobic compounds belonging to the class of 2-methyl-1, 4 naphthoquinone derivatives. In humans, the main source of Vitamin K is represented by phyloquinone (Vitamin K1) contained in vegetables such as spinach, cabbage, and broccoli and in fruits such as kiwi and bananas. Cooking food does not significantly reduce Vitamin K content. Vitamin K2 (menaquinone) is produced by intestinal bacteria, but it is uncertain whether it contributes to the requirement of this vitamin in humans. The adequate intake (AI) of Vitamin K ranges from 2.0 and 2.5 µg/day in infants of 0-6 months and 7-12 months, respectively, while the amount of this vitamin raises to 30-55 µg/day in infants between 1 and 8 years. In adults, the AI ranges from 90 and 120 µg in women and men, respectively [23].

But why is Vitamin K so important in preventing bleeding?

Vitamin K induces the carboxylation of glutamate residues of proteins (Gla residues) to form gamma-carboxyglutamate. The carboxylated Gla residues in this way can bind Ca ions, which are essential for the coagulability of four factors of the coagulation cascade (II, VII, IX, and X) on the phospholipid membranes [24] (**Fig. 4**). This step is also crucial for the function of other proteins involved in the control of blood coagulation activity, such as Protein C, Protein S, and Protein Z, natural anticoagulants. However, Vitamin K is also important for the function of proteins involved in bone metabolism, such as osteocalcin, periostin, and the matrix Gla protein [25]. The discovery of Vitamin K was crucial for the development of Vitamin K antagonists, i.e. coumarins anticoagulants, whose use as antithrombotic drugs is still widespread in the world [26].

Fibrin formation

Fibrin formation starts from fibrinogen, a 340-kDa protein that circulates at concentrations of

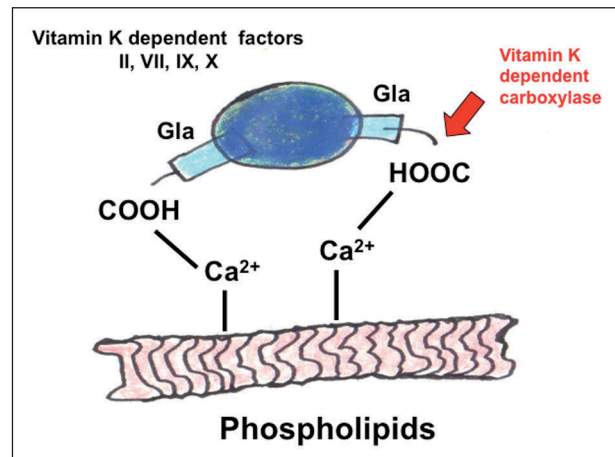


Figure 4. The role of Vitamin K in blood coagulation. Vitamin K induces the carboxylation of glutamate residues of proteins (Gla residues) to form gamma-carboxyglutamate. The carboxylated Gla residues in this way can bind Ca ions, which are essential for the coagulability of four factors of the coagulation cascade (II, VII, IX, and X) on the phospholipid membranes.

1.5-4.5 mg/mL. It consists of two D and one E domains, which contain a pair of three disulfide-linked chains (A α , B β , and γ). The passage from fibrinogen to fibrin is a crucial step in blood coagulation because it is essential for stopping the bleeding but also in the pathophysiology of vascular obstruction and thrombosis. Thrombin, coming from the activation of its zymogen, prothrombin, cleaves fibrinopeptides A and B from the N-terminal portions of the A α and B β chains of fibrinogen, thus inducing the formation of fibrin monomers, which in turn go toward polymerization [27]. At the next non-enzymatic step, monomeric fibrin self-assembles spontaneously to form fibrin oligomers that lengthen to make two-stranded protofibrils. They put themselves both laterally and longitudinally to form fibers that branch to yield a three-dimensional network [28]. The final outcome is the fibrin I formation characterized by the interaction of one E domain of one molecule with the D domain of another, generating a fibrillary pattern so that a clot starts to develop. Furthermore, thrombin activates Factor XIII, a transglutaminase, which is able to cross-link fibrin at lysine residues of adjacent fibrin monomers, as well as the a-chains of opposing monomers to form D-dimer and a-polymers, respectively [29] (**Fig. 5**). This phenomenon, in the end, gives stability and strength to the clot (fibrin II). Also, platelets aggregate and Red Blood Cells participate since they become incorporated into its structure [30].

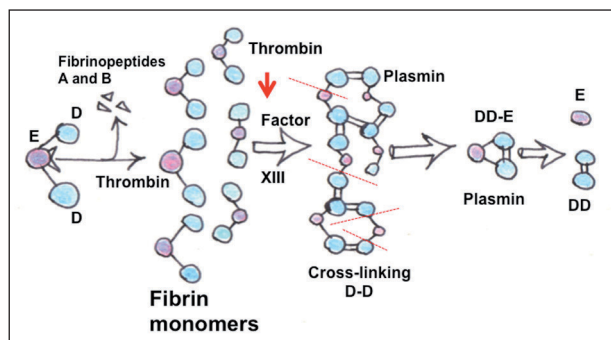


Figure 5. Fibrin formation and D-dimer production. Factor XIII cross-links D domain to each other. Plasmin cannot lyse the cross-linked D domain. D-dimer are then released into the circulation.

Fibrinolysis

Fibrinolysis deals with the dissolution of the clot being a defensive system devoted to prevent unnecessary accumulation of intravascular fibrin [31]. Plasmin is the pivotal protease of fibrinolysis and is activated from plasminogen by either tissue Plasminogen Activator (tPA) or urokinase Plasminogen Activator (uPA). tPA is synthesized and released by endothelial cells, while uPA is produced by monocytes and urinary epithelium [32]. They have a short half-lives (4-8 minutes) because of the presence of potent inhibitors such as Plasminogen Activator Inhibitors (PAI-1 and PAI-2) [33]. The role of these inhibitors is devoted to avoid an upregulation of plasmin activity. Another important regulation of fibrinolysis comes from the action of α_2 antiplasmin, which forms a 1:1 stoichiometric complex with plasmin; in consequence, both become inactive. However, α_2 antiplasmin does not work as long as plasmin is bound to fibrin, thus allowing an adequate fibrinolysis [34]. Finally, Thrombin Activated Fibrinolysis Inhibitor (TAFI) is a carboxypeptidase that exerts its function removing C-terminal lysine residues of fibrin, so limiting the number of plasminogen binding sites. It thus reduces plasmin generation, reinforcing clot stability and strength. Thrombin, either alone or in complex with TM, is the main activator of TAFI [35]. When thrombin production is low, such as in haemophilia, TAFI is consequently reduced, thus contributing to form a weak clot more susceptible to fibrinolysis [36].

Once plasmin starts to work, the release of the Fibrin Degradation Products (FDPs) begins [37]. When fibrin is cleaved by plasmin, the resulting D-dimer fragment reflects the degree of thrombosis and plasmin activity [38]. Plasmin induces the lysis

of the fibrin network into soluble fragments, such as (DD)E: a complex formed by of D-dimer coming from cross-linked adjacent D domains (DD) non-covalently bound to fragment E. Plasmin causes proteolysis of fragment E from the (DD)E complex [39]. D-dimer then circulates in plasma with a half-life of about 8 hours, cleared by the kidneys and the reticuloendothelial system [40]. Since D-dimer comes from cross-linked fibrin, it can be considered a marker of activation of the coagulative and fibrinolytic systems [38].

Conclusions

The hemostatic system is complex, but in the last years more knowledge has been reached on this topic, especially in the field of bleeding and thrombosis. To know how this system works is of paramount importance for understanding the mechanisms underlying several pathological conditions such as inflammation, cancer, bleeding and thrombosis.

Another important point is that referred to drugs employed either as hemostatic or antithrombotic agents.

Declaration of interest

The Authors declare that there is no conflict of interest.

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