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Update / Atualização

A cell-based model of coagulation and its implications

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The concept of a coagulation cascade describes the biochemical interactions of the coagulation factors, but it is flawed as a model of the in vivo hemostatic process. Hemostasis requires both platelet and fibrin plug formation at the site of vessel injury and that the procoagulant substances activated in this process remain at the site of injury. This control of blood coagulation is accomplished as the procoagulant reactions only exist on specific cell surfaces to keep coagulation from spreading throughout the vascular system. A model of coagulation that better explains bleeding and thrombosis in vivo created after considering the critical role of cells. The cell-based model of hemostasis replaces the traditional "cascade" hypothesis, and proposes that coagulation takes place on different cell surfaces in four overlapping steps: initiation, amplification, propagation and termination. The cell-based model allows a more thorough understanding of how hemostasis works in vivo, and sheds light on the pathophysiological mechanism for certain coagulation disorder.

***Keywords**: Blood coagulation; Blood coagulation factors; Blood coagulation disorders/ physiopathology; Blood platelets/metabolism; Hemostasis; Thromboplastin; Protein C; Protein S; Antithrombins; Anticoagulants*

Introduction

The classic coagulation cascade, proposed in 1964 by Macfarlane[(1)] and Davie & Ratnoff [(2)] is described in numerous articles and textbooks. Although this model has limitations and can not satisfactorily explain all phenomena related to *in vivo* hemostasis, it has been accepted for almost fifty years. This conventional model referred to as "cascade" was proposed to explain the physiology of blood clotting, according to which, coagulation occurs through sequential proteolytic activation of pro-enzymes by plasma proteases, resulting in the formation of thrombin, which then breaks up the fibrinogen molecule to fibrin monomers. This proposal divides coagulation in an extrinsic pathway (involving blood elements and elements that are usually not found in the intravascular space) and an intrinsic pathway (started by components that exist in the intravascular space), which converge to a common pathway with the activation of factor X (FX). In the extrinsic pathway, plasma factor VII is activated in the presence of its cofactor, tissue factor (TF), forming the factor VIIa/TF complex (FVIIa/ TF), which is responsible for the activation of factor X. In the intrinsic pathway, activation of factor XII occurs when blood comes into contact with a surface containing negative electrical charges. This process, called "contact activation", requires the presence of other plasma components:

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prekallikrein (a serine protease) and high-molecular-weight kininogen (a non-enzymatic cofactor). Activated factor XII activates factor XI, which in turn activates factor IX. Factor IXa in the presence of factor VIII activated by traces of thrombin and in the presence of calcium ions (tenase complex) activates coagulation factor X, generating thrombin and subsequently fibrin formation.[3-5]

Although the concept of coagulation "cascade" was a successful model and a significant advance in the understanding of coagulation, more recent clinical and experimental observations show that the cascade hypothesis does not fully reflect *in vivo* hemostasis.[6]

In recent years, the deficiencies of this classic model have become evident. For example, deficiencies of factor XII, prekallikrein or of high-molecular-weight kininogen prolong the activated partial thromboplastin time (aPTT) but do not cause bleeding.[7] On the other hand, deficiency of factor IX causes hemophilia B and severe clinical bleeding. The "cascade" model does not explain why the activation of factor X by the extrinsic pathway is not able to compensate for impairment of the intrinsic pathway due to a lack of factor VIII (hemophilia A) or factor IX (hemophilia B).[8] Moreover, the degree of aPTT prolongation in hemophilia patients does not necessarily predict the extent of the bleeding. As was pointed out by Hoffman[6,9] the activity of the extrinsic pathway of hemophilia patients is normal, as is evidenced by the prothrombin time (PT) even though aPTT is prolonged and they have pronounced tendency to bleed. This has raised the following question: why does the extrinsic pathway not compensate for the dysfunction of the intrinsic pathway, in other words, why do hemophiliacs bleed?

Many researchers acknowledge that the cascade model has serious flaws in relation to the physiological coagulation model and that the extrinsic and intrinsic pathways can not operate as independent and redundant pathways as suggested by this model.[6,10,11] It has also been recognized in previous studies on coagulation, that the cells play an important role in this process and that normal hemostasis is not possible without tissue factor being together with cells and platelets. So it seems logical that by replacing the role of cells by phospholipid vesicles in the *in vitro* PT and aPTT tests ignores the active role of such cells in the *in vivo* conditions.[10]

Given these questions and some key observations, it seems that the classical coagulation model needs to be revisited as it can not answer several important questions related to the conditions of individuals with certain hemostatic disorders. Thus, a model for hemostasis based on cell surfaces was developed to replace the classical coagulation cascade model. This new model suggests an interaction of coagulation factors with specific cell surfaces and seems to be able to explain many issues hitherto not explained by the traditional coagulation cascade.

Coagulation cascade model based on cell surfaces

Over the last 15 years, major advances have occurred in the field of hemostasis in light of important discoveries related to *in vivo* blood coagulation, triggered by cells that express TF on their surface. TF is a transmembrane protein that acts as a receptor and cofactor for factor VII; it is normally expressed in cells outside the vasculature.[12,13] This understanding has resulted in questioning the true role of the intrinsic pathway in *in vivo* hemostasis. Evidence suggests that, although factor XII deficiency does not result in excessive bleeding, its paucity does not protect against thrombosis.[7,9]

Recently, a model based on cell surfaces was proposed in which hemostasis requires activated procoagulant substances that remain at the site of the injury and are involved in the formation of platelet plug and fibrin plugs. In this new model, the blood clotting process is initiated by contact of TF to the bloodstream. The TF is not constitutively expressed in endothelial cells but is present in the membranes of cells around blood vessels, such as in smooth muscle cells and fibroblasts. Thus, the TF is exposed to the bloodstream due to damage to the endothelium and surrounding cells or by the activation of endothelial cells or monocytes.[12] Considerable evidence suggests that TF is also present as cellular microparticles in blood from fragmented membranes of different cell types, such as leucocytes, endothelial cells and platelets. These microparticles may play an important role in thrombotic processes. It is well known that the FVIIa/TF complex activates not only factor X but also factor IX. Studies show that this complex is essential to start *in vivo* clotting.[10]

The current understanding of the hemostatic process considers the interrelationship of physical, cellular and biochemical processes in a series of stages or phases and not as two pathways (intrinsic and extrinsic) as was believed. The stages of initiation, amplification, propagation and termination illustrate the intriguing process that ensures the circulation of blood in a liquid form restricted to the vascular bed. These four phases, summarized in Table 1, compose the current coagulation theory based on cell surfaces.

Initiation phase

The initiation phase of the coagulation process occurs when cells that express TF on their surface are exposed to blood components at the site of injury.[4,8] The TF, once bound to FVII in blood, quickly activates it forming the FVIIa/TF complex which is responsible for the activation of small amounts of FIX and FX.[11,14] FXa associated with its cofactor, FVa, forms a complex called prothrombinase on the surface of cells that express TF. The FV can be activated by FXa or

non-coagulating proteases, resulting in FVa which is necessary for the prothrombinase complex. This complex transforms small amounts of prothrombin (Factor II) to thrombin; amounts too small to complete the fibrin clot formation process, but this is critical in the coagulation amplification phase (Figure 1).[6,8,14] It is believed that the reactions responsible for initiating coagulation are constantly taking place outside the blood vessels of healthy subjects. It has been proven that clotting factors, including FVII, FX and prothrombin, are able to cross the space between tissues, i.e., they can leave the vessel as these factors have been detected in the lymph. The amount of each substance outside of vessels is primarily dependant on molecular size.[6] Based on these observations it has been suggested that the initiation route remains continuously active, with small quantities of activated factors being produced at baseline. Thus, small amounts of thrombin are continuously produced outside the vascular space, independent of vascular injury.[11] Hence, it is assumed that a little activity in the TF pathway occurs all the time in the extravascular space. The coagulation process proceeds to the amplification phase only when there is vascular damage, i.e. when platelets and FVIII (linked to von Willebrand factor) come into contact with extravascular tissue where they adhere to cells expressing TF.[9,10]

Table 1. Summary of current cell-based model of coagulation

Coagulation phases			
Initiation	Amplification	Propagation	Termination
Vascular endothelium and circulating blood cells are disturbed; Interaction of plasma-derived activated factor VII with tissue factor	Thrombin activates platelets, cofactors V and VIII and factor XI on the surface of platelets	Production of large amounts of thrombin, the formation of a stable buffer at the site of injury and interruption of blood loss	Process of coagulation is restricted to prevent thrombotic occlusion of the intact areas of vessels

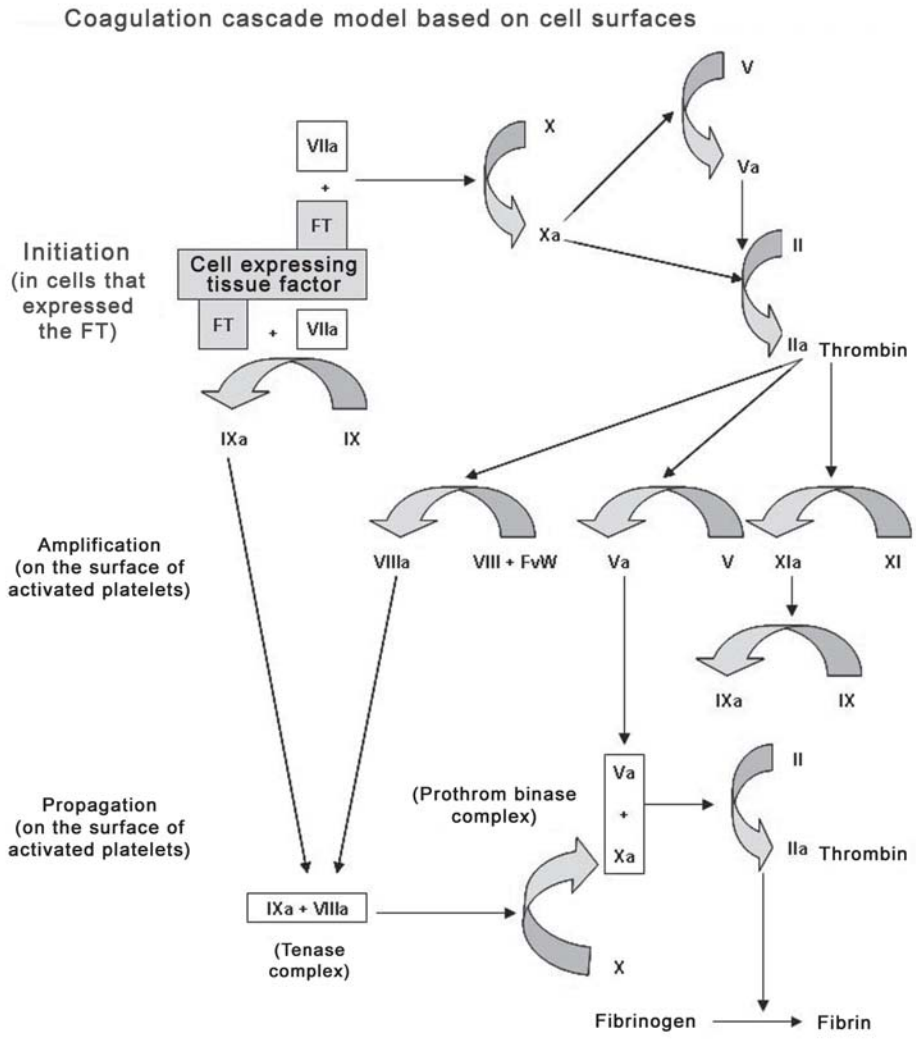

Figure 1. Illustration of the coagulation model based on cell surfaces comprising the phases of initiation, amplification and propagation. Tissue factor (TF), activated (a). Translated and adapted from Vine, AK. Recent advances in haemostasis and thrombosis. Retina. 2009;29(1):1-7[8]

Amplification phase

Due to their large sizes, platelets and FVIII bound to von Willebrand factor (vWF) only pass to the extravascular compartment when there is vascular injury. When a vessel is injured, platelets leave the vessel, bind to collagen and other components of the extracellular matrix at the site of injury, where they are partially activated and form a platelet plug responsible for primary hemostasis.[5,15] At this point, small amounts of thrombin produced by cells that express TF can interact with platelets and the FVIII/vWF complex. This begins the hemostatic process that

culminates in the formation of stable fibrin, secondary hemostasis, which consolidates the initial platelet plug.[4,11,15]

This small amount of thrombin generated by cells expressing TF has several important functions with the main one being the maximum activation of platelets, which make receptors and binding sites available to activated coagulation factors. As a result of this activation, the permeability of platelet membranes is altered, allowing the entry of calcium ions and the release of chemotactic substances that attract clotting factors to their surface in addition to releasing partially activated FV.[4,14] Another function of the thrombin formed during the initiation phase, is the activation of the FV and FVIII cofactors on the surface of activated platelets. The FVIII/vWF complex is dissociated, allowing vWF to mediate platelet adhesion and aggregation at the site of injury. In addition, small amounts of thrombin activate FXI (FXIa) on the platelet surface during this phase. Activation of FXI by thrombin on the platelet surface explains why FXII is not required for normal hemostasis. Simultaneously, due to chemotactic mechanisms, these factors are attracted to the surface of platelets where they rapidly began the propagating phase (Figure 1). [8,9,11,14]

Propagation phase

The propagation phase is characterized by the migration of large numbers of platelets to the site of injury and the production of tenase complex and prothrombinase on the surface of activated platelets.[8] First, the FIX activated during the initiation phase can now bind to FVIIIa on the platelet surface forming the tenase complex. An extra amount of FIXa can also be produced by FXIa bound to platelets. As FXa can not effectively move from cells expressing TF to activated platelets, a large amount of FXa must be generated directly on the platelet surface by the FIXa/FVIIIa complex.[9] Finally, FXa rapidly associates with FVa bound to the platelet during the amplification phase resulting in the formation of the prothrombinase complex which converts large amounts of prothrombin into thrombin. This is responsible for the cleavage of fibrinogen into fibrin monomers that, in turn, polymerize to consolidate the platelet plug (Figure 1).[10]

Termination phase

Once a fibrin clot is formed at the lesion, the clotting process must be limited to the injury site to prevent thrombotic occlusion of the vessel. Four natural anticoagulants are involved to control the spread of coagulation activation; tissue factor pathway inhibitor (TFPI), protein C (PC), protein S (PS) and antithrombin (AT).

TFPI is a protein secreted by the endothelium, which forms a quaternary complex, TF/FVIIa/FXa/TFPI, which inactivates activated factors and limits coagulation.[7] Protein C and S are two other natural anticoagulants that have the ability to inactivate the procoagulant FVa and FVIIIa cofactors.[16] PC is a vitamin K-dependent plasma glycoprotein, whose synthesis, when activated, promotes proteolysis of Va and VIIIa cofactors.[17] PC is activated by thrombin, which is bound to the transmembrane protein, thrombomodulin (TM) on the surface of intact endothelial cells.[18] The activity of PC is increased by another cofactor inhibitor, which is also vitamin K-dependent, PS. In human plasma, approximately 30% of PS circulates as free protein, consisting of the fraction that functions as a cofactor for activated PC.[19,20] Another natural anticoagulant, AT, inhibits the activity of thrombin and other serine proteases such as FIXa, FXa, FXIa and FXIIa.[21] Endothelial cells produce a variety of glycosaminoglycans, which serve as high-affinity binding sites for AT, which are crucial for quick inactivation of thrombin.[8,22]

Advantages of the new model of coagulation

This new model of hemostasis is able to explain some clinical aspects of hemostasis that the classical cascade model does not. This new model gives a better understanding of the *in vivo* coagulation process and is more consistent with the clinical observations of several coagulation disorders.

Implications of the new coagulation model for laboratory tests

Traditionally, screening methods included an assessment of blood clotting using the activated partial thromboplastin time (aPTT), which analyzes the intrinsic pathway, and prothrombin time (PT), which evaluates the extrinsic coagulation pathway.[9,23] The new coagulation model based on cell surfaces has shown that the extrinsic and intrinsic pathways are not redundant. The extrinsic pathway operates on the surface of cells expressing TF to initiate and intensify the clotting process and the components of the intrinsic pathway operate on the surface of activated platelets to produce large amounts of thrombin which result in the formation and stabilization of the fibrin clot. Thus, the PT assesses the levels of procoagulant involved in the initiation phase of coagulation, while the aPTT assesses the levels of procoagulant generated during the propagation phase that are consequently involved in the production of a large amount of thrombin on the surface of activated platelets.[11]

It is important to note that the coagulation cascade model and common coagulation tests do not reflect the

complexity of *in vivo* hemostasis. Nevertheless, coagulation tests have sensitivity to detect deficiencies of one or more coagulation factors and are therefore efficient to define changes in the coagulation factors of patients with a tendency to bleed. It is important to remember that no test is able to provide a complete and reliable profile of the hemostatic function; the new cell-based hemostasis model incorporates the active participation of cellular structures to direct and control the process and none of the available tests include cellular components.

According to Monroe & Hoffman[11] although the PT and aPTT do not reflect the role of inhibitors and do not necessarily identify the clinical risk of bleeding, they are not without value; it is important to emphasize that the interpretation of the results should be considered in light of all clinical conditions.

Implications of the new model of coagulation in hemophilia

When compared to the traditional cascade model, the new model based on cell surfaces better explains the pathophysiological mechanisms involved in hemophilia. For example, the cascade model does not explain why the extrinsic pathway seems unable to produce sufficient amounts of FX to partially compensate for deficiencies of FVIII or FIX. In other words, one of the intriguing questions cited by Hoffman[6] refers to the lack of a plausible explanation for the fact that the activation of FX by the TF/VIIa complex fails to substitute FXa that is typically generated by the FIXa/FVIIIa complex. The model based on the cell surfaces does not suggest that the FXa generated by the TF/VIIa complex is inadequate in hemophilia, but is "poorly" expressed on the cell surface. The FIXa/FVIIIa complex activates FX on the surface of platelets during the propagation phase; however, the TF/FVIIa may only produce FXa on the surface of cells expressing TF, as it is unable to move to the surface of activated platelets. Furthermore, it is important to mention that there are two very efficient FXa inhibitors in plasma, TFPI and AT. At normal plasma levels, TFPI and AT inhibit FXa so quickly and effectively that the half-life of FXa is one minute or less in the fluid phase. Therefore, the FXa that remains in cells expressing TF must be relatively well protected from inhibitors but any FXa that diffuses from the cell surface is rapidly inhibited.[6,9] The cell-based coagulation model suggests that hemophilia is specifically a deficiency in the generation of FXa on the surface of platelets resulting in a low production of thrombin on the surface of platelets. Hemophilia patients have relatively normal coagulation initiation and amplification phases and are able to form the initial platelet plug at the site of bleeding. However, they are incapable of generating an amount of thrombin on the platelet surface sufficient to stabilize the fibrin clot.[6]

Final considerations

The cell-based coagulation model of hemostasis enables a better understanding of the clinical problems seen in some coagulation disorders by emphasizing the central role of specific cell surfaces to control the hemostatic process. This model provides a potentially more accurate representation of the hemostatic process and facilitates the interpretation of coagulation tests and the pathophysiological mechanisms of clotting disorders such as hemophilia. Finally, the new cell-based coagulation theory can be considered a step forward in the understanding of major clinical events related to hemostasis. However, further investigations are being conducted in order to improve our understanding of the complex hemostatic mechanisms.

Resumo

O conceito da cascata da coagulação descreve as interações bioquímicas dos fatores da coagulação, entretanto, tem falhado como um modelo do processo hemostático in vivo. A hemostasia requer a formação de um tampão de plaquetas e fibrina no local da lesão vascular, bem como a permanência de substâncias procoagulantes ativadas nesse processo no sítio da lesão. O controle da coagulação sanguínea é realizado por meio de reações procoagulantes em superfícies celulares específicas e localizadas, evitando a propagação da coagulação no sistema vascular. Uma análise crítica do papel das células no processo hemostático permite a construção de um modelo da coagulação que melhor explica hemorragias e tromboses in vivo. O modelo da coagulação baseado em superfícies celulares substitui a tradicional hipótese da "cascata" e propõe a ativação do processo de coagulação sobre diferentes superfícies celulares em quatro fases que se sobrepõem: iniciação, amplificação, propagação e finalização. O modelo baseado em superfícies celulares permite um maior entendimento de como a hemostasia funciona in vivo e esclarece o mecanismo fisiopatológico de certos distúrbios da coagulação.

Descritores: *Coagulação sanguínea; Fatores de coagulação sanguínea; Transtornos da coagulação sanguínea/ fisiopatologia; Plaquetas/metabolismo; Hemostasia; Tromboplastina; Proteína C; Proteína S; Antitrombinas; Anticoagulantes*

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