

Coagulation 2006: A Modern View of Hemostasis

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HOW WELL DO WE REALLY UNDERSTAND COAGULATION?

In the 1960s two groups proposed a waterfall or cascade model of coagulation composed of a sequential series of steps in which activation of one clotting factor led to the activation of another, finally leading to a burst of thrombin generation [1,2]. Each clotting factor was believed to exist as a proenzyme that could be converted to an active enzyme.

The original cascade models were subsequently modified to include the observation that some procoagulants were cofactors and did not possess enzymatic activity. The coagulation process is now often outlined in a Y-shaped scheme, with distinct intrinsic and extrinsic pathways initiated by factor XII (FXII) and FVIIa/tissue factor (TF), respectively, as outlined in Fig. 1. The pathways converge on a common pathway at the level of the FXa/Fva (prothrombinase) complex. The coagulation complexes are generally noted to require phospholipid and calcium for their activity. This scheme was not actually proposed as a literal model of the hemostatic process in vivo. The lack of any other clear and predictive concept of physiologic hemostasis, however, has meant that most physicians and students of coagulation viewed the cascade as a model of physiology. This view has been reinforced by the fact that screening tests of the adequacy of the extrinsic pathway (prothrombin time [PT]) and the intrinsic pathway (activated partial thromboplastin time [aPTT]) are often treated as though they are predictive of clinical bleeding.

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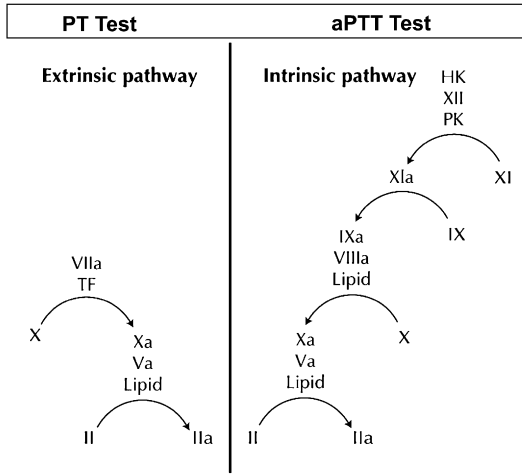


Fig. 1. The extrinsic and intrinsic pathways in the cascade model of coagulation. These two pathways are conceived as each leading to formation of the factor Xa/Va complex, which generates thrombin. *Lipid* indicates that the reaction requires a phospholipid surface. These pathways are assayed clinically using the prothrombin time (PT) and activated partial thromboplastin time (aPTT), respectively. HK, high molecular weight kininogen; PK, prekallikrein.

Although the cascade concept of coagulation was treated as though it were a model of coagulation *in vivo*, many people recognized that the intrinsic and extrinsic systems could not operate *in vivo* as independent and redundant pathways as implied by this model. It was clear that even though deficiencies of each of the factors in the intrinsic pathway could have equally long aPTT values, they had dramatically different risks of hemorrhage. Deficiencies of FXII are not associated with significant hemorrhage, deficiencies of FXI might or might not be associated with hemorrhage, but deficiencies of factors VIII and IX are consistently associated with hemorrhage.

The key observation that the FVIIa/TF complex activated not only FX but also FIX [3], suggested that the pathways were linked. Other important observations led to the conclusion that activity of the FVIIa/TF complex is the major initiating event in hemostasis *in vivo* [4,5]. It was still not clear why an intact extrinsic pathway could not compensate for the lack of FIX or VIII in hemophilia, however.

CAN AN EMPHASIS ON THE ROLE OF CELLS IMPROVE OUR UNDERSTANDING OF COAGULATION?

It was recognized from the earliest studies of coagulation that cells were important participants in the coagulation process. Of course, it is clear that normal hemostasis is not possible in the absence of platelets. In addition, TF is an integral membrane protein and thus its activity is normally associated with cells. Because different cells express different levels of pro- and anticoagulant

proteins and have different complements of receptors for components of hemostasis, it is logical that simply representing the cells involved in coagulation as phospholipid vesicles may overlook the important contributions of cells in directing hemostasis *in vivo*. The authors' studies in a cell-based experimental model of coagulation [6–8] and the existing literature led us to propose [9] that hemostasis actually occurs in a step-wise process, regulated by cellular components *in vivo*, as outlined in the following sections.

Step 1: Initiation of Coagulation on TF-bearing Cells

The goal of hemostasis is to produce a platelet and fibrin plug to seal a site of injury or rupture in the blood vessel wall. This process is initiated when TF-bearing cells are exposed to blood at a site of injury.

TF is a transmembrane protein that acts as a receptor and cofactor for FVII. Once bound to TF, zymogen FVII is rapidly converted to FVIIa through mechanisms not yet completely understood but possibly involving FXa or noncoagulation proteases. The resulting FVIIa/TF complex catalyzes activation of FX and activation of FIX. The factors Xa and IXa formed on the TF-bearing cells have distinct and separate functions in initiating blood coagulation [7]. The FXa formed on the TF-bearing cell interacts with its cofactor Va to form prothrombinase complexes and generates a small amount of thrombin on the TF cells (Fig. 2A). By contrast, the FIXa activated by FVIIa/TF does not act on the TF-bearing cell and does not play a significant role in the initiation phase of coagulation. If an injury has occurred and platelets have adhered near the site of the TF-bearing cells, the FIXa can diffuse to the surface of nearby activated platelets. It can then bind to a specific platelet surface receptor [10], interact with its cofactor, FVIIIa, and activate FX directly on the platelet surface.

Most of the coagulation factors can leave the vasculature and their activation peptides are found in the lymph [11]. It is likely, therefore, that most (extravascular) TF is bound to FVIIa even in the absence of an injury, and that low levels of FIXa, FXa, and thrombin are produced on TF-bearing cells at all times. This process is kept separated from key components of hemostasis by an intact vessel wall, however. The very large components of the coagulation process are platelets and FVIII bound to multimeric von Willebrand factor (vWF). These components normally only come into contact with the extravascular compartment when an injury disrupts the vessel wall. Platelets and FVIII-vWF then leave the vascular space and adhere to collagen and other matrix components at the site of injury.

Step 2: Amplification of the Procoagulant Signal by Thrombin Generated on the TF-bearing Cell

Binding of platelets to collagen or by way of vWF leads to partial platelet activation. The coagulation process is most effectively initiated, however, when enough thrombin is generated on or near the TF-bearing cells to trigger full activation of platelets and activation of coagulation cofactors on the platelet surface in the amplification step (as illustrated in Fig. 2B). Although this amount of thrombin may not be sufficient to clot fibrinogen, it is sufficient to initiate

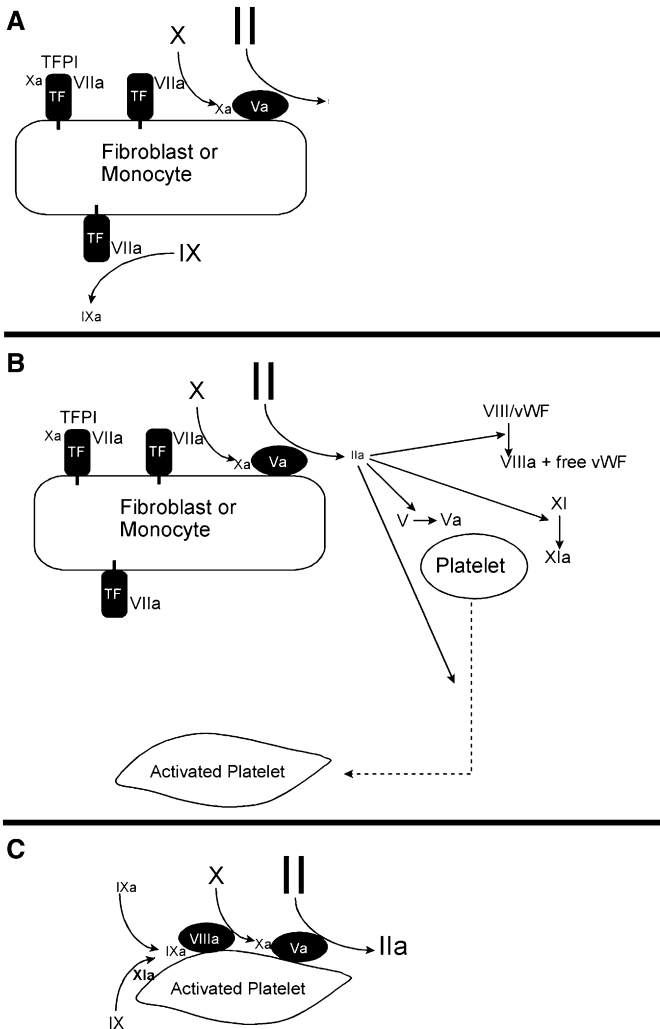


Fig. 2. Steps in a cell-based model of coagulation. (A) Initiation occurs on the TF-bearing cell as activated FX combines with its cofactor, FVa, to activate small amounts of prothrombin. (B) The small amount of thrombin generated on the TF-bearing cell amplifies the procoagulant response by activating cofactors, factor XI, and platelets. (C) The large burst of thrombin required for effective hemostasis is formed on the platelet surface during the propagation phase.

events that prime the clotting system for a subsequent burst of platelet surface thrombin generation. Experiments using a cell-based model have shown that minute amounts of thrombin are formed in the vicinity of TF-bearing cells exposed to plasma concentrations of procoagulants, even in the absence of platelets. The small amounts of FVa required for prothrombinase assembly on TF-bearing cells are activated by FXa [12] or by noncoagulation proteases

produced by the cells [13] or are released from platelet that adhere nearby. The small amounts of thrombin generated on the TF-bearing cells are responsible for [8,14]: (1) activating platelets, (2) activating FV, (3) activating FVIII and dissociating FVIII from VWF, and (4) activating FXI. The activity of the FXa formed by the FVIIa/TF complex is restricted to the TF-bearing cell, because FXa that dissociates from the cell surface is rapidly inhibited by TFPI or AT in the fluid phase. In contrast to FXa, FIXa can diffuse to adjacent platelet surfaces because it is not inhibited by TFPI and is inhibited much more slowly by AT than is FXa.

Step 3: Propagation of Thrombin Generation on the Platelet Surface

Platelets play a major role in localizing clotting reactions to the site of injury because they adhere and aggregate at the sites of injury where TF is also exposed. They provide the primary surface for generation of the burst of thrombin needed for effective hemostasis during the propagation phase of coagulation (Fig. 2C). Platelet localization and activation are mediated by vWF, thrombin, platelet receptors, and vessel wall components, such as collagen [15].

Once platelets are activated, the cofactors Va and VIIIa are rapidly localized on the platelet surface [6]. As noted above, the FIXa formed by the FVIIa/TF complex can diffuse through the fluid phase and also bind to the surface of activated platelets. Likewise, FXI also binds to platelet surfaces and is activated by the priming amount of thrombin [14,16], bypassing the need for FXIIa. The platelet-bound FXIa can activate more FIX to IXa. Once the platelet tenase complex is assembled, FX from the plasma is activated to FXa on the platelet surface. FXa then associates with FVa to support a burst of thrombin generation of sufficient magnitude to produce a stable fibrin clot.

The large amount of thrombin generated on the platelet surface is responsible for stabilizing the hemostatic clot in more ways than just promoting fibrin polymerization. In fact, most of the thrombin generated during the hemostatic process is produced after the initial fibrin clot is formed. The platelet-produced thrombin also stabilizes the clot by: (1) activating FXIII [17], (2) activating TAFI [18], (3) cleaving the platelet PAR-4 receptor [19], and (4) being incorporated into the structure of the clot.

The role of FXI in hemostasis has been a point of some controversy, because even severe FXI deficiency does not result in a hemorrhagic tendency comparable to that seen in severe FVIII or IX deficiency. This discrepancy can be explained if FXI is viewed as an enhancer or booster of thrombin generation. FXIa activates additional FIXa on the platelet surface to supplement FIXa/FVIIIa complex formation and enhance platelet surface FXa and thrombin generation. FXI thus is not essential for platelet-surface thrombin generation, as are FIX and FVIII, and its deficiency does not compromise hemostasis to the degree seen in FIX and FVIII deficiency.

Our knowledge of the platelet contribution to thrombin generation continues to evolve. There is evidence that there is more than one population

of activated platelets, one of which has been referred to as COAT (Collagen And Thrombin stimulated) platelets [20]. These platelets have enhanced thrombin-generating ability because of enhanced binding of both tenase and prothrombinase component [21,22]. The *in vivo* relevance of these findings is not yet clear, but it may be that the greatest procoagulant activity is generated on platelets that have bound to collagen matrix and also been exposed to thrombin. Once the exposed collagen matrix is covered by a platelet/fibrin layer, additional platelets that accumulate are not activated to the COAT state, thus tending to damp down the procoagulant signal as the area of the wound is occluded by a hemostatic clot.

Although each step of the cell-based model has been depicted as an isolated set of reactions, including initiation, amplification, and propagation, they should be viewed as an overlapping continuum of events. For example, thrombin produced on the platelet surface early in the propagation phase may initially cleave substrates on the platelet surface and continue to amplify the procoagulant response, in addition to leaving the platelet and promoting fibrin assembly.

The cell-based model of coagulation shows us that the extrinsic and intrinsic pathways are not redundant. Let us consider the extrinsic pathway to consist of the FVIIa/TF complex working with the FXa/Va complex and the intrinsic pathway to consist of FXIa working with the complexes of factors VIIIa/IXa and factors Xa/Va as illustrated in Fig. 3 (from Ref. [23]). The extrinsic pathway operates on the TF-bearing cell to initiate and amplify coagulation. By contrast, the intrinsic pathway operates on the activated platelet surface to produce the burst of thrombin that causes formation and stabilization of the fibrin clot.

FIBRINOLYSIS

Even as the fibrin clot is being formed in the body, the fibrinolytic system is being initiated to disrupt it. The final effector of the fibrinolytic system is plasmin, which cleaves fibrin into soluble degradation products. Plasmin is produced from the inactive precursor plasminogen by the action of two plasminogen activators: urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA). The PAs are in turn regulated by plasminogen activator inhibitors (PAIs). Plasminogen is found at a much higher plasma concentration than the PAs. The availability of the two PAs in the plasma therefore generally determines the extent of plasmin formation. tPA release from endothelial cells is provoked by thrombin and venous occlusion [24]. tPA and plasminogen both bind to the evolving fibrin polymer. Once plasminogen is activated to plasmin it cleaves fibrin at specific lysine and arginine residues, resulting in dissolution of the fibrin clot.

Thrombin-activatable inhibitor of fibrinolysis (TAFI) is a zymogen that can be activated (TAFIa) by thrombin or plasmin [18]. As fibrin is degraded by plasmin, C-terminal lysines are exposed that enhance activation of additional plasminogen to plasmin. TAFIa removes the C-terminal lysines from fibrin and thereby inhibits the cofactor activity of fibrin for plasminogen activation.

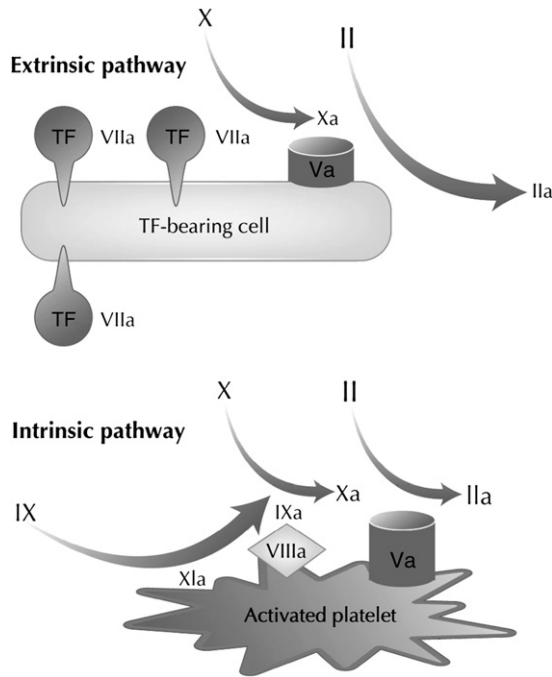


Fig. 3. The extrinsic and intrinsic pathways in the cell-based model of coagulation. The role of the cell-based extrinsic pathway (top) is to act on the TF-bearing cell to generate the small amounts of thrombin (factor IIa) involved in initiating coagulation. The role of the cell-based intrinsic pathway (bottom) is to act on the platelet surface to generate the burst of thrombin needed to form a stable fibrin clot. TF, tissue factor.

Fibrinolysis is essential for removal of clots during the process of wound healing and for removing intravascular clots that might otherwise be manifest as thrombosis. Intravascular deposition of fibrin is also associated with the development of atherosclerosis. An effective fibrinolytic system therefore tends to protect against the chronic process of atherosclerotic vascular disease and the acute process of thrombosis. Conversely, defects of fibrinolysis increase the risk for atherothrombotic disease. For example, elevated levels of plasminogen activator inhibitor-1, an inhibitor of fibrinolysis, are associated with an increased risk for atherosclerosis and thrombosis [25] as are decreased levels of plasminogen [26]. The effectiveness of hemostasis *in vivo* depends not only on the procoagulant reactions but also on the fibrinolytic process.

WHAT DOES ALL THIS MEAN FOR CLINICAL LABORATORY TESTING?

It should be clear from the preceding discussion that our commonly used clinical coagulation tests do not really reflect the complexity of hemostasis *in vivo*.

That does not mean that the PT and aPTT are useless. We just need to understand what they can and cannot tell us. These screening coagulation tests are abnormal when there is a deficiency of one or more of the soluble coagulation factors. They do not tell us what the risk for clinical bleeding will be. Two patients who have identical aPTT values can have drastically different risks of hemorrhage. All of our common coagulation tests including the PT, aPTT, thrombin clotting time, fibrinogen levels, and coagulation factor levels tell us something about the plasma level of soluble factors required for hemostasis. Their clinical implications must be evaluated by the ordering physician. Just because the PT and aPTT are within the normal range it does not follow that the patient is at no risk for bleeding. Conversely, a mild elevation in these clotting times does not mean that the patient is at risk for bleeding after an invasive procedure.

Many whole blood coagulation tests are jockeying for position as a means of evaluating overall hemostatic status in selected clinical settings. Although whole blood tests have the advantage that they may reflect the contributions of platelets to the hemostatic process, they still do not reflect the contributions of the TF-bearing cells and local tissue conditions. Any laboratory test requires skilled interpretation and clinical correlation in evaluating the true risk for bleeding.

WHAT CAUSES BLEEDING IN PREVIOUSLY NORMAL PATIENTS?

Many patients who experience significant hemorrhage do not have an underlying bleeding tendency that can be identified before a bleeding episode. Bleeding following surgical or accidental trauma or during a medical illness is often associated with the development of an acquired coagulopathy. The hallmark of coagulopathy is microvascular bleeding, which means oozing from cut surfaces and minor sites of trauma, such as needle sticks. Microvascular bleeding can lead to massive blood loss.

Causes of coagulopathic bleeding include consumption of coagulation factors and platelets, excessive fibrinolysis, hypothermia, and acidosis.

Consumption of Coagulation Components

We normally think of disseminated intravascular coagulation (DIC) when we talk of consumption. Clotting factors and platelets can also be consumed during appropriate physiologic attempts at hemostasis, however. In this case it is appropriate to replace the depleted factors with transfusion therapy.

DIC can be much more complicated to manage [27]. The mainstay of treatment is to treat the underlying disorder, such as sepsis. In early or mild/compensated DIC administration of low-dose heparin may be considered to control the procoagulant response to inflammation, infection, or malignancy. In more severe or advanced DIC, however, replacement therapy may be necessary to treat the bleeding tendency associated with depletion of coagulation factors and platelets.

Excessive Fibrinolysis

The process of fibrinolysis is initiated whenever coagulation is initiated. When attempts at hemostasis are unsuccessful, a significant amount of fibrinolytic activity may still be generated and thwart subsequent efforts at hemostasis. Fibrinolytic inhibitors have thus proven to be useful in some circumstances.

Hypothermia

Many patients become hypothermic during medical illness or following surgical or accidental trauma [28]. Hypothermia can directly interfere with the hemostatic process by slowing the activity of coagulation enzymes. Less well recognized is the finding that platelet adhesion and aggregation is impaired even in mild hypothermia [29]. In hypothermic coagulopathic patients, raising the core temperature can have a beneficial effect on bleeding by improving platelet function and coagulation enzyme activity.

Acidosis

Acidosis can have an even more profound effect on procoagulant function than hypothermia, although the two metabolic abnormalities often coexist. A drop in the pH from 7.4 to 7.2 reduces the activity of each of the coagulation proteases by more than half [30]. Acidosis should be considered as a possible contributor to coagulopathic bleeding in medical and surgical patients.

WHAT HAPPENS AFTER THE BLEEDING STOPS?

Once hemostasis is completed the process of wound healing can begin. The hemostatic plug must be stable enough to maintain hemostasis, yet be removed as the tissue defect is permanently closed. Fibrinolysis is accomplished by the action of plasmin, probably in concert with other leukocyte proteases. The neutrophils that initially accumulate at a site of injury are replaced over the course of a few days with macrophages that engulf and degrade cellular debris and components of the fibrin clot. The macrophages also secrete cytokines and growth factors that facilitate the migration of fibroblasts and endothelial cells into the wound site. In the case of a skin wound, the dermis is replaced by highly cellular and vascular granulation tissue, while the surface epithelium proliferates and migrates from the margins to cover the surface of the wound. Many of the activities involved in wound healing are influenced by thrombin. Thrombin plays a major role in platelet activation and degranulation. Several key cytokines modulating wound healing are released from activated platelets, including transforming growth factor beta (TGF β 1) and platelet-derived growth factor (PDGF). The amount and rate of thrombin generated during hemostasis influences the initial structure of the fibrin clot—the framework on which cell migration takes place. In addition, thrombin has chemotactic and mitogenic activities for macrophages, fibroblasts, smooth muscle cells and endothelial cells. Generation of the right amount of thrombin during the coagulation process not only may be essential for effective hemostasis but also may set the stage for effective wound healing.

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