

Review Article

A Cell-based Model of Hemostasis

Maureane Hoffman, Dougald M. Monroe III

Pathology and Laboratory Medicine Service, Durham VA and Duke University Medical Centers,
Durham, NC, USA, and Division of Hematology/Oncology, Department of Medicine,
The University of North Carolina, Chapel Hill, NC, USA

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Summary

Based on our work and that of many other workers, we have developed a model of coagulation *in vivo*. Many workers have demonstrated mechanisms by which cells can influence the coagulation process. Nonetheless, the prevailing view of hemostasis remains that the protein coagulation factors direct and control the process with cells serving primarily to provide a phosphatidylserine containing surface on which the procoagulant complexes are assembled. By contrast, we propose a model in which coagulation is regulated by properties of cell surfaces. This model emphasizes the importance of specific cellular receptors for the coagulation proteins. Thus, cells with similar phosphatidylserine content can play very different roles in hemostasis depending on their complement of surface receptors. We propose that coagulation occurs not as a "cascade", but in three overlapping stages: 1) initiation, which occurs on a tissue factor bearing cell; 2) amplification, in which platelets and cofactors are activated to set the stage for large scale thrombin generation; and 3) propagation, in which large amounts of thrombin are generated on the platelet surface. This cell based model explains some aspects of hemostasis that a protein-centric model does not.

Modeling Hemostasis

A model is a way of conceptualizing and understanding a complicated system. A good model should be simple enough to understand, yet complicated enough to accurately reflect the process it was designed to represent. The purpose of this paper is to present a model of coagulation which focuses on the roles of specific cell surfaces in controlling the coagulation process. This paper is intended to be part of a series in which different views of the coagulation process are presented.

A "Cascade" or "Waterfall" model was a great advance in our understanding of coagulation, and was originally proposed by two groups nearly simultaneously (1, 2). This model was subsequently refined to the scheme shown in Fig. 1 as more was learned about the biochemistry of the coagulation factors. Every model highlights aspects of a process that are viewed as critical by the modeler. The cascade model of coagulation resulted from work that was aimed at

elucidating the identity, function and interactions of the individual procoagulant proteins. It accurately represents the overall structure of the coagulation process as a series of proteolytic reactions. Each protease cleaves and activates the subsequent protease in the series. The Cascade model also included the recognition that anionic phospholipid, especially phosphatidylserine, was required for the assembly and optimal function of most of the coagulation complexes (3). This information was absolutely critical to understanding the coagulation reactions. However, the viewpoint that is implicit in this concept of coagulation is that the role of cells, especially platelets, is primarily to provide anionic phospholipid for coagulation complex assembly.

The coagulation "Cascade" models very well the screening coagulation laboratory tests, the prothrombin time (PT) and activated partial thromboplastin time (aPTT), which correspond to the extrinsic and intrinsic pathways. However, it is clearly inadequate to explain the pathways leading to hemostasis *in vivo*. As this model currently exists, it is inconsistent with clinical observations in several key respects. If there are separate "intrinsic" and "extrinsic" coagulation pathways *in vivo* as shown in the Cascade model, why can the activation of factor X by the extrinsic (f.VIIa/TF-initiated) pathway not compensate for a lack of factor VIII or IX in hemophiliacs. In fact the activation of hemostasis by an "intrinsic" pathway *in vivo* must be questioned anyway, since deficiency of f.XII, high molecular weight kininogen (HMK) or pre-kallikrein (PK) does not cause a clinical bleeding tendency. Of course, it is clear that some components of the "intrinsic" pathway, f.VIII and f.IX, are essential for hemostasis, since their deficiency leads to hemophilia. We also know that deficiencies of f.X, f.V and f.VII lead to serious clinical bleeding syndromes. By contrast, f.XI deficiency is much less predictable in causing a bleeding diathesis and leads to a less severe clinical picture than deficiency of f.IX or f.VIII. Therefore, we (4-6) and others (7, 8) concluded that there are very likely not separate "extrinsic" and "intrinsic" pathways operating under normal conditions *in vivo*, and the overall model of coagulation required rethinking.

A key step in understanding how the "extrinsic" and "intrinsic" pathways might be interdependent *in vivo* was the recognition that the f.VIIa/TF complex can activate f.IX as well as f.X (9). Also important was the finding that thrombin could directly activate factor XI on a charged surface (10). Once it was learned that activated platelets could provide a surface for activation of f.XI by thrombin under physiologic conditions (11, 12), it was clear why factor XII, HMK and PK might not be required for hemostasis. This led to a concept of hemostasis in which tissue factor (TF) was the primary physiologic activator. It was still not clear why direct activation of f.X by f.VIIa/TF could not compensate, at least partially, for a lack of factors IX or VIII. It was hypothesized that tissue factor pathway inhibitor (TFPI) inhibited f.VIIa/TF before it could produce enough f.Xa for hemostasis (7). These modifications to the cascade model reflect the evolution of our understanding of how the

Correspondence to: Dr. Maureane Hoffman, Pathology & Laboratory Medicine Service (113), Durham VA Medical Center, Durham, NC 27705, USA – Tel.: +1 919 286-6925; Fax: +1 919 286 6818; E-mail: maureane@med.unc.edu

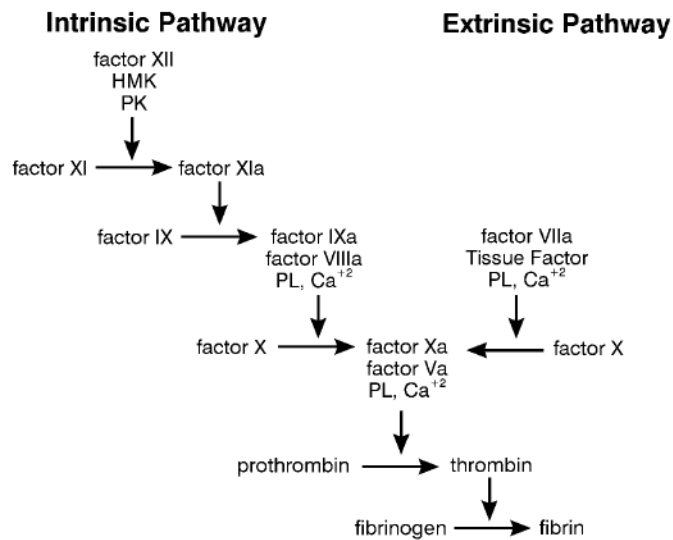


Fig. 1 The cascade model of coagulation. The "intrinsic" and "extrinsic" pathways are reflected in the clinical laboratory tests aPTT and PT, respectively

coagulation proteins interact during hemostasis. However, the prevailing concepts of coagulation still viewed the cellular components as primarily providing a phospholipid surface on which the proteins could express their activity.

There is now a significant body of work from many researchers showing that different cell surfaces have very different properties as related to the coagulation process, even if the cells have similar membrane lipid composition. Coagulant properties result from expression of a variety of cell features, including protein receptors, that localize components of the coagulation system to specific cell surfaces. Our work has led us to focus on how the localization of the coagulation reactions to different cell surfaces serves to control the coagulation process. This potentially represents a paradigm shift – from a concept that views coagulation as being controlled by the levels and kinetics of the coagulation proteins to one that considers the process to be controlled by the cellular components. We believe that a clearer understanding

of the role of cells has allowed us to answer some of the unanswered questions in hemostasis and develop a cell-based model that reflects the pathways of hemostasis in vivo.

A Cell-Based Model of Hemostasis

We view hemostasis as occurring in three (overlapping) phases. The *initiation* of coagulation takes place on TF-bearing cells, such as the fibroblast illustrated in Fig. 2. If the procoagulant stimulus is sufficiently strong, enough factors Xa, IXa and thrombin are formed to successfully initiate the coagulation process. *Amplification* of the coagulant response occurs as the "action" moves from the TF-bearing cell to the platelet surface. The procoagulant stimulus is amplified as platelets adhere, are activated and accumulate activated cofactors on their surfaces. Finally, in the *propagation* phase, the active proteases combine with their cofactors on the platelet surface – the site best

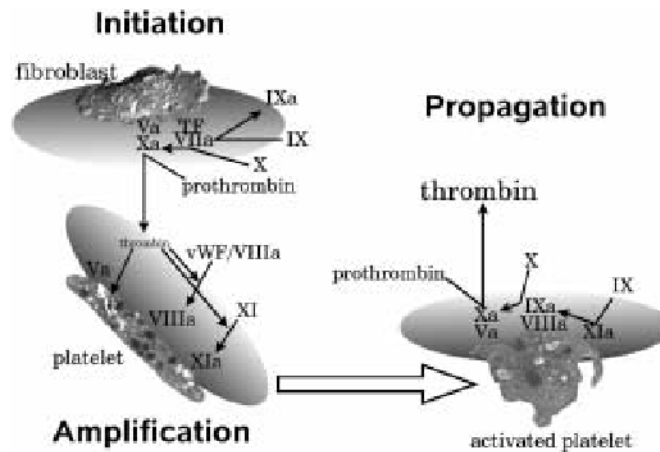


Fig. 2 A cell-based model of coagulation. The three phases of coagulation occur on different cell surfaces: Initiation on the tissue factor-bearing cell; Amplification on the platelet as it becomes activated; and Propagation on the activated platelet surface

adapted to generate hemostatic amounts of thrombin. The activity of the procoagulant complexes produces the burst of thrombin generation that results in fibrin polymerization.

Inappropriate coagulation is prevented by several mechanisms. The inactivation and propagation steps are localized on different cell surfaces. The plasma protease inhibitors localize the reactions to cell surfaces by inhibiting active proteases that diffuse into the fluid phase. Finally, endothelial cells express active antithrombotic features that prevent coagulation from being initiated in the intact endothelium.

Initiation

TF is the primary physiologic initiator of coagulation. It is structurally unrelated to the rest of the coagulation proteins and is an integral membrane protein (13, 14). Therefore, TF remains localized to the membrane of the cell in which it was synthesized. It is expressed on a variety of extravascular cells under normal conditions, and can also be expressed by blood monocytes and endothelial cells in inflammatory states. There are now data suggesting that TF-containing membrane vesicles may bind to platelet surfaces in an evolving thrombus (15). The source and physiologic role of such vesicles remain uncertain, but it is clear that normal circulating unactivated platelets neither contain nor express TF. It is important that the initiating and propagating steps are normally sequestered to different cell surfaces to limit the undesired activation of coagulation. Bringing f.VIIa/TF activity into close proximity to activated platelet surfaces is a key step in effective initiation of hemostatic coagulation (or thrombosis).

During the process of hemostasis, a break in the vessel wall allows plasma to come into contact with TF-bearing extravascular cells. Factor VII in plasma binds tightly to cellular TF and is rapidly activated by coagulation (16) and non-coagulation proteases. The f.VIIa/TF complex activates both f.X and f.IX. Factor Xa can activate plasma f.V (17), as can non-coagulation cellular proteases (18, 19). The f.X activated by the f.VIIa/TF complex is rapidly inhibited by TFPI or ATIII if it leaves the protected environment of the cell surface. However, the f.Xa that remains on the cell surface can combine with f.Va to produce small amounts of thrombin (20) which plays an important role in subsequently activating platelets and f.VIII during the amplification phase.

Amplification

Damage to the vasculature allows platelets as well as plasma to come into contact with extravascular tissues. Platelets adhere to extravascular matrix components at the site of injury. The process of binding to matrix proteins partially activates platelets, as well as localizing them near a site of TF exposure. Small amounts of thrombin generated on TF-bearing cells amplify the initial procoagulant signal by enhancing platelet adhesion (21), fully activating platelets and activating factors V, VIII and XI (20). Thus, thrombin acts on the platelet surface to "set the stage" for procoagulant complex assembly.

Thrombin is a potent platelet activator via its protease-activated receptors (PAR) (22). During activation, platelets release f.V from alpha granules onto their surfaces in a partially activated form (23). Factor V(a) is then fully activated by thrombin or f.Xa (17).

Some of the thrombin bound to non-PAR receptors, such as GPIb/IX, remains active and can activate other coagulation factors on the platelet surface. von Willebrand factor (vWF)/f.VIII binds to platelets and is efficiently cleaved by thrombin to activate f.VIII and release it from vWF (24). The f.VIIIa remains bound to the platelet

surface. Now that the platelets have been activated and have activated cofactors V and VIII bound to their surfaces, assembly of the procoagulant complexes and large-scale thrombin generation begins.

Propagation

During the propagation phase the "tenase" and "prothrombinase" complexes are assembled on the platelet surface, and large-scale thrombin generation takes place. Platelets express high affinity binding sites for f.IX(a) (25), f.X(a) (26), and f.XI (27). We believe these receptors play important roles in coordinating assembly of the coagulation complexes.

The "tenase" (f.VIIIa/IXa) complexes assemble when f.IXa reaches the platelet surface. The f.IXa can diffuse to platelet surfaces from its site of activation on TF-bearing cells, since it is not rapidly inhibited by ATIII or other plasma protease inhibitors. In addition, plasma f.XI can bind to activated platelets, facilitating its activation by thrombin (11). The f.XIa can then provide additional f.IXa directly on the platelet surface. The f.IXa/VIIIa complexes activate f.X on the platelet surface where the resulting f.Xa can move directly into a complex with its cofactor, f.Va, as illustrated in Fig. 3. The platelet surface f.Xa/Va complexes can now produce the burst of thrombin necessary to form a hemostatic fibrin clot.

Mechanisms of Procoagulant Localization

As implied in the above model of coagulation, we believe that specialized cellular features are critical to localizing, and thereby controlling, the coagulation process. The plasma proteins are available to any cell that comes into contact with blood. The cells can "choose" which of the proteins they will utilize by binding them to selective sites on their surfaces. Cells have features that adapt them to specialized roles in hemostasis, including cell surface expression of protein receptors, lipid composition of the outer leaflet of the plasma membrane, cell surface expression of glycosaminoglycans and synthesis or storage of coagulation proteins.

Procoagulant Mechanisms of TF-Bearing Cells

Since TF is accepted as the primary initiator of coagulation, it has been assumed that the amount of TF exposure will predict whether or not clotting will occur. While TF expression is necessary for initiation of coagulation or thrombosis, it is not sufficient. Additional factors are

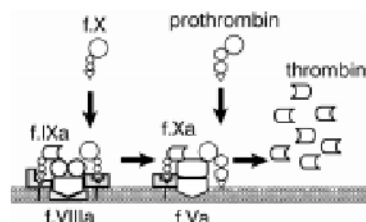


Fig. 3 Assembly and activity of the "tenase" and "prothrombinase" complexes during the propagation phase of coagulation on the activated platelet surface. Factor X is activated in the relatively protected environment of the platelet surface. It can move directly into a complex with platelet-bound factor Va without leaving the platelet surface and risking inhibition by plasma protease inhibitors.

1. Platelets adhere to extra-vascular matrix components at the site of injury.

Anchor Name: activated platelets only at the site of vascular injury [Agency

Switzerland

m.waldis@fatzerimbach.ch]

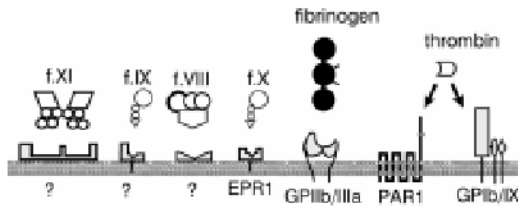


Fig. 4 Specific receptor coordinate procoagulant activity on the platelet surface. Platelets have very different properties than other cells, or phospholipid vesicles in supporting the activity of the coagulation reactions. The differences are largely due to the particular complement of receptors (or binding sites) present on the platelet surface

involved in determining whether an effective procoagulant "signal" will be produced (20). For example, a cell that expresses TF but can not support prothrombinase assembly is much less efficient in initiating coagulation than is a cell with the same amount of TF that can also support thrombin generation (5). A good example of this concept is the ability of malignant cells to promote thrombosis *in vivo*. Many malignancies express TF, but their tendency to cause thrombosis does not necessarily correlate with TF activity (28). The divergent abilities of different tumor cells to activate prothrombin (29) might account for some of the discrepancy.

Coagulation Factor Localization on Platelets

Platelets are, of course, the cell type most closely associated with the coagulation process. They provide the surface on which most of the thrombin required for clot formation is generated. In spite of the fact that they are well adapted to assemble coagulation complexes on their membrane surface, they do not express TF, the primary physiologic initiator of coagulation. The normal process of hemostatic coagulation can proceed when cellular TF is brought into close proximity to activated platelets and a source of coagulation factors.

One very important feature of platelets is their ability to adhere to sites of injury and become activated by one or more pathways. Upon

activation, they undergo a number of changes that allow them to serve as a surface for assembly and activity of the coagulation complexes. Platelets do not express significant amounts of phosphatidylserine (PS) when in the unactivated state. Activation results in an increase in PS exposure on the membrane outer leaflet (30) from virtually none to up to 12% (31). This PS exposure is necessary but not sufficient to account for the procoagulant properties of platelets.

In addition to triggering PS exposure, platelet activation also leads to secretion of partially activated f.V from alpha granules (23) and activation of surface receptors (32, 33). It seems to us extremely likely that the binding of all of the coagulation factors that normally act on platelet surfaces is mediated by specific high-affinity interactions with protein receptors or binding sites. In this context we will use the term receptor to mean a protein binding site, that may or may not transduce a signal to the cell. Several platelet adhesive receptors have been extensively characterized, but the enumeration and characterization of non-lipid binding sites for coagulation factors is in its infancy. A number of specific platelet surface receptors have been postulated to contribute to coordination of the coagulation reactions as illustrated in Fig. 4. There is only indirect evidence for the existence of most of them.

Both unactivated and activated platelets have several receptors or binding sites for thrombin: Glycoprotein (GP) Ib/IX (34), protease-activated receptors (PAR) (22) and possibly others. Thrombin PARs have been cloned and well characterized (22). This type of receptor clearly transduces a signal for activation to the platelet (35). GPIb/IX is well characterized as the receptor for vWF. It also binds thrombin (36) and plays a role in platelet activation by thrombin (37). However, it is likely that one or more additional high affinity binding sites for thrombin are present on platelets (38-40).

In addition to serving as one of the platelet surface thrombin receptors, the GPIb/IX complex is the primary binding site for vWF. The binding sites on GPIb/IX for vWF/f.VIII and thrombin are distinct (36), thus both proteins can be bound simultaneously. Rather than mediating platelet activation by thrombin, GPIb/IX seems primarily to localize thrombin to the surface of the platelet, where it can activate other procoagulant factors and cofactors as in Fig. 5.

In its capacity as a receptor for vWF, GPIb/IX promotes adhesion of unactivated platelets at sites of vascular injury. Adhesion of platelets through GPIb/IX and vWF promotes at least partial platelet activation. In addition to its role as an adhesive protein, vWF is the carrier protein for plasma factor VIII. Binding of vWF to GPIb/IX localizes f.VIII to the platelet surface, where it is most efficiently activated by thrombin. By this mechanism the relatively unstable activated form of f.VIII is produced directly on the surface where its activity is needed to support the coagulation reactions. The f.VIIIa remains localized on the platelet surface by binding to a distinct platelet binding site (41).

Platelet Receptors for Coagulation Factors

Unlike GPIb/IX and GPIIb/IIIa, receptors for most of the coagulation factors have not been identified unequivocally, and the evidence for their existence is inferential. However, it seems likely that such receptors coordinate assembly of the factors into functional complexes and may even participate in formation of a "supercomplex" of the f.IXa/f.VIIIa and f.Xa/Va complexes.

While it has not yet been sequenced or cloned, there is strong functional evidence that a protein binding site exists for factor IX/Xa on platelets (32, 42, 43). It is "activated" upon platelet activation, localizes factor IXa to the platelet surface, and facilitates activation of factor X

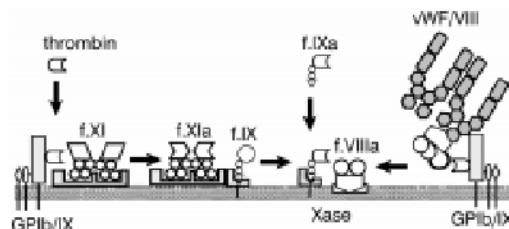


Fig. 5 Thrombin "sets the stage" for the propagation phase of coagulation by activating factors VIII and XI on the platelet surface. Binding to GPIb/IX localizes thrombin to the platelet surface. The bound thrombin retains the ability to cleave macromolecular substrates, including factors VIII and XI. Factor VIII is bound to its carrier protein, vWF. Factor VIII/vWF is localized to the platelet surface also by binding to GPIb/IX. Factor VIII is released from vWF once it is activated by thrombin, and the resulting factor VIIIa can bind to its own binding site on platelets. Factor XI is also localized to the platelet surface by binding to a specific receptor. This binding facilitates its activation by thrombin

by f.IXa. There is evidence for a specific, non-lipid activation-induced binding site for f.VIII(a) on platelets as well (41, 44).

There is evidence that a protein receptor exists on platelets for factor X(a) and may serve to coordinate assembly of the prothrombinase (f.Xa/Va) complex (45, 46). The receptor for f.Xa has been proposed to be effector protease receptor-1 (EPR-1), a protein that has been cloned, sequenced and characterized as a signal-transducing receptor for f.Xa on other cell types (47).

Factor XI can bind to platelets via high molecular weight kininogen (27), but also binds directly to a small number of high affinity sites on platelets through its apple 3 domain (48). It is the latter interaction that facilitates f.XI activation by thrombin on activated platelets (11, 12).

One coagulation protein that does not appear to have a specific non-lipid binding site on platelets is factor VIIa (49, 50), since it normally does not express its activity on platelets, but rather on tissue factor-bearing cells. Therefore, the binding and activity of f.VIIa on platelets appears similar to that on synthetic lipid vesicles.

Endothelial Cells Localize Clot Formation to the Site of Injury

While TF-bearing cells and platelets have specialized procoagulant functions, vascular endothelial cells have specialized anti-coagulant features. In a number of ways endothelial cells can be considered to be close relatives of platelets and can express a number of the same receptors in a regulated fashion, including GPIb (51), vWF (52), f.V, thrombin receptor (PAR), and P-selectin (53). In addition, recent evidence has shown that endothelial cells can be repopulated from bone marrow progenitor cells. However, endothelial cells have several unique mechanisms to contain clot formation to a site of injury and prevent thrombosis.

Thrombin that escapes into the circulation from a site of injury must be controlled to prevent it from clotting fibrinogen in the systemic circulation. It is either inhibited by ATIII in the plasma or binds to thrombomodulin (TM) on intact endothelial cells (54). TM is a receptor for thrombin. TM is expressed at high levels on endothelial cells, especially in the microvasculature. Upon binding to TM, the specificity of thrombin is changed (55). It no longer clots fibrinogen or activates platelets, but becomes much more effective at activating Protein C.

Thrombin in complex with TM serves to activate Protein C on the endothelial surface. Protein C is localized to endothelial surfaces by an endothelial Protein C receptor (EPCR-1), that facilitates its activation by thrombin/TM (56). Activated Protein C (APC) forms a complex with Protein S. The APC/Protein S complex cleaves and inactivates any f.Va and F.VIIIa that has been activated on endothelial cell surfaces. This prevents the formation of additional procoagulant enzymes at sites where a healthy, intact endothelial lining layer is present. This process is illustrated in Fig. 6. Thus, thrombin is effectively changed from a pro-coagulant to an anti-thrombotic when it is localized to an endothelial cell surface that expresses TM.

In addition to TM, endothelial cells also express other important anti-thrombotic surface features. Endothelial cells enhance inactivation of coagulation factors by ATIII and TFPI (57), presumably due to the presence of heparan-like glycosaminoglycans on their surface. Endothelial cells also express cell-surface ADPase activity (CD39) (58). This enzyme metabolizes ADP released from activated platelets, resulting in blockade of the aggregation response when the platelets are in close proximity to healthy endothelium.

Under normal conditions vascular endothelial cells are highly specialized to be actively anti-thrombotic. However, they can respond

to injury or inflammation by altering expression of receptors and, thereby, becoming less anti-thrombotic (59). Injury or inflammatory cytokines can induce endothelial cells to decrease expression of TM, and increase expression of TF and surface adhesion molecules. This is probably an adaptive defense mechanism that facilitates hemostasis at sites of injury. However, these mechanisms can contribute to thrombosis in a number of disease states, such as atherosclerosis, thrombophlebitis and vasculitis.

Localization Determines the Fate of Coagulation Factors

Receptors and binding sites play an important role in localizing coagulation proteins to specific cell surfaces. However, the plasma protease inhibitors also play a major role in localizing the coagulation activity. Only a portion of the total amount of an activated factor will be bound to cell surfaces. The activated factors that are surface-bound are relatively protected from plasma protease inhibitors, while proteases in the solution phase are more rapidly inactivated. Thus, under usual conditions, activated factors tend to express their activity on or very near the surface on which they are activated. Those that do not remain localized on a cell surface are inhibited and prevented from catalyzing coagulation reactions in the systemic circulation. When this control mechanism is overwhelmed, disseminated intravascular coagulation ensues.

An Emphasis on Localization Might Explain Some Clinical Problems that a Purely Enzymatic Approach Does Not

An emphasis on the primary role of the coagulation proteins in controlling coagulation suggests that one should be able to understand the physiology and pathophysiology of hemostasis if only one had a sufficiently good understanding of the enzymology and kinetics of the individual reactions. So far this has not proven to be true. We feel that there are several important clinical problems that an emphasis on the cellular control of coagulation has let us understand.

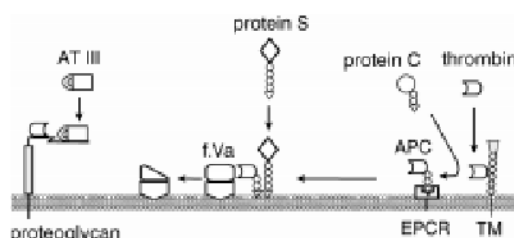


Fig. 6 Endothelial cells express surface features that mediate their anti-thrombotic nature. The specialized complement of receptors on endothelial cells are geared toward rendering their surface unsuitable for large-scale thrombin generation. Thrombomodulin (TM) and the endothelial protein C receptor (EPCR) facilitate localization of protein C and thrombin to the endothelial surface, where the thrombin/TM complex activates protein C. In combination with its cofactor, protein S, activated protein C (APC) cleaves and inactivates f.Va (and f.VIIIa, not shown). Proteoglycans on the endothelial membrane bear heparan sulfate that binds and enhances the protease inhibiting activity of ATIII

Why Do Hemophiliacs Bleed?

As introduced earlier in this review, the cascade model of coagulation does not explain why the “extrinsic” pathway can not produce enough f.Xa to (at least partially) compensate for a lack of factor VIII or IX, i.e. why can’t f.Xa generated by f.VIIa/TF substitute for f.Xa generated by f.IXa/VIIIa? It has been hypothesized that this is because TFPI shuts off the f.VIIa/TF pathway before it can make enough f.Xa to support generation of hemostatic amounts of thrombin.

While inhibition of the TF pathway by TFPI might contribute to the bleeding tendency in hemophilia, we believe the problem in hemophilia can be better understood by looking elsewhere. We hypothesize that the problem in hemophilia is not that enough f.Xa isn’t made, but that it is made ON THE WRONG CELL SURFACE. Factor Xa made on a TF-bearing cell has a very hard time making its way to the platelet surface without being inhibited by either ATIII or TFPI. These two inhibitors, at their normal plasma levels, inhibit f.Xa so efficiently that it has a half life of a minute or less in the solution phase. By contrast, factor IXa is inhibited much less rapidly by ATIII and not at all by TFPI. Therefore, f.IXa can move from the TF-bearing cell to the activated platelet surface to form the platelet tenase complex. In order for f.Xa to be incorporated efficiently into prothrombinase complexes, it must be formed on the platelet surface (i.e. by f.IXa/VIIIa) in close proximity to f.Va. Thus, we propose that in hemophilia there is a failure specifically of platelet-surface f.X activation leading to a failure of platelet-surface thrombin generation.

Why Does F.VIIa Provide Hemostasis in Hemophilia?

We believe that thinking of hemophilia as a failure of platelet-surface f.Xa generation also allows us to understand the efficacy of high-dose f.VIIa in promoting hemostasis in hemophiliacs. It has been well documented that f.VIIa can lead to hemostasis in hemophiliacs, however, the mechanism by which it acts has been a matter of ongoing debate. The doses of f.VIIa required for efficacy produce plasma levels that are several orders of magnitude greater than the Kd for binding of f.VIIa to TF. This led many to conclude that it is unlikely that f.VIIa works via TF. However, it is also well recognized that f.VIIa has very little proteolytic activity in the absence of its cofactor. We have worked hard to convince the reader that cells do much more than provide phospholipid for hemostasis. However, in this case we believe that the platelet surface behaves as a PS-containing lipid surface in supporting the activity of f.VIIa. We have shown that f.VIIa can bind to activated platelets (but not unactivated platelets) with an affinity similar to its affinity for synthetic PS-containing lipid vesicles (50). Once bound to the activated platelet surface, f.VIIa can activate f.X (50). The amount of f.Xa generated by this mechanism is sufficient to support near normal levels of thrombin generation in a experimental model of factor IX and VIII deficiency (49, 60). This finding is consistent with our model of coagulation in which platelet surface f.Xa generation is necessary to support platelet surface prothrombinase assembly. Since we believe that platelet surface thrombin generation fails in hemophilia, restoration of platelet surface thrombin generation by high levels of f.VIIa is a plausible mechanism of action. In further support of a platelet surface mechanism of action for high dose f.VIIa is our finding that the levels of f.VIIa that must be attained for efficacy in vivo correlate very well with the levels needed for restoration of platelet surface thrombin generation in vitro. A platelet surface mechanism would also explain why high dose f.VIIa is effective in establishing hemostasis in patients with thrombocytopenia and platelet function

defects. We have found that in models of these conditions, the addition of high levels of f.VIIa enhances the amount of thrombin generated on each platelet.

Recently, the activity of f.VIIa was studied in a coagulation model using plasma concentrations of purified coagulation factors with reactions initiated by TF relipidated into synthetic phospholipid vesicles. These workers showed that, in the presence of plasma levels of zymogen f.VII, the lag before initiation of coagulation was shortened by the addition of recombinant f.VIIa (61). The authors hypothesized that this mechanism could account for the clinical efficacy of high dose f.VIIa in hemophilia. However, we find that when cell-associated TF is used to initiate coagulation, the presence of zymogen f.VII does not extend the lag period, nor does f.VIIa shorten the lag before initiation of coagulation. Therefore, it seems unlikely that this mechanism can account for the efficacy of f.VIIa in vivo.

Our findings do not preclude any contribution from a TF-mediated mechanism of action for high dose f.VIIa therapy. However, a platelet surface mechanism is better able to explain the clinical efficacy of high dose f.VIIa therapy. In addition, localization of f.VIIa to activated platelets also accounts for the lack of significant thrombotic complications to high dose f.VIIa therapy. Localization to the activated platelet surface would tend to localize procoagulant activity to the site where it is appropriate.

Is Thrombosis Mechanistically Different than Hemostasis?

This is an important question in the development of antithrombotic strategies. In general, every antithrombotic therapy is also anticoagulant and has a significant risk of bleeding associated with it. We tend to think of thrombosis as hemostasis occurring at the wrong place – the implication being that the two processes are mechanistically identical. However, if thrombosis is coagulation on the “wrong” cell surface, it implies that different cellular components may be involved than when hemostatic coagulation takes place. This possibility leads us to hypothesize that there are mechanistic differences inherent in thrombosis occurring on endothelial surfaces that might be exploited in developing antithrombotic strategies. Targeting therapeutics to endothelial surfaces or away from platelet surfaces might prove to be a clinically useful strategy.

It is worthwhile to keep in mind that the endothelial cells from different vascular beds have different coagulant properties. Thus, it is possible that therapeutics could be targeted to a vascular bed that had a higher risk of thrombosis in a given patient population. For example, patients with activated Protein C resistance have a much greater risk of venous than arterial thrombosis. If it were possible to target antithrombotic agents to the venous circulation, it might lead to better efficacy in those patients with less risk of bleeding. In addition to different vascular beds having different properties, inflammation can alter the properties of endothelial cells by changing expression of pro- and anti-coagulant molecules as well as adhesive receptors. Thus, it might be possible to target therapeutics to sites of inflammation – which are also more likely to be sites of thrombosis.

In summary, we believe that thinking of coagulation as occurring on specific cell surfaces can help us understand better the mechanism of normal hemostasis as well as understanding the mechanisms by which hemostasis fails in pathologic conditions.

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