

Fig. 5.1. Hemostasis in health. Normal hemostasis is maintained by the numerous and complex interactions of blood vessels, platelets, coagulation pathways, and the fibrinolytic system that lead to the formation and resolution of a secondary hemostatic plug after vascular injury. The following events occur with blood vessel damage:

- Vasoconstriction reduces blood loss, and activated endothelial cells express both prothrombotic functions to limit bleeding and antithrombotic functions to limit clotting.
- Platelets adhere to exposed subendothelium, spread to patch the defect, release products that activate other platelets, and aggregate to form a primary hemostatic plug. Their secretory products also help maintain vasoconstriction and promote coagulation, and their membranes are an important source of phospholipid to accelerate coagulation.
- The surface-induced and TF coagulation pathways are activated, leading to the production of thrombin and subsequent conversion of fibrinogen to fibrin within the primary hemostatic plug. This forms a stable secondary hemostatic plug that controls bleeding. Thrombin also activates platelets, endothelial cells, and TAFI.
- When coagulation pathways are activated, fibrinolysis is also. Fibrinolysis helps control the extent of coagulation by breaking down fibrin, thus contributing to the formation of an appropriate secondary hemostatic plug and promoting eventual removal of the plug to maintain normal blood flow.

Abnormalities of any component of this system can upset the balance and lead to either hemorrhage or thrombosis.

Fig. 5.2. Coagulation cascade. The coagulation cascade begins with activation of the TF (extrinsic) or surface-induced (intrinsic) pathways, and results in the formation of cross-linked fibrin by the common pathway (beginning with factor X). *Bold arrows* represent major coagulation pathways in vivo.

- **TF pathway:** It was originally thought to require extravascular activation and was therefore named *extrinsic*. This pathway is initiated by TF released from, or exposed on, damaged tissue or activated monocytes, macrophages, and possibly endothelial cells. These cells can be activated by endotoxin and certain inflammatory cytokines. Cell membrane TF binds to factor VII (and VIIa) in the presence of fCa^{2+} , and the resulting activated TF-VIIa complex rapidly activates factor X (common pathway) and factor IX (surface-induced pathway) in the presence of fCa^{2+} and phospholipid (PL). TFPI rapidly inactivates TF-VIIa, but not until thrombin is generated through the common pathway. Thrombin activates factor XI and the amplification pro-cofactors V and VIII for sustained production of factor Xa via the surface-induced pathway.
- **Surface-induced pathway:** It was originally considered the pathway activated by intravascular (intrinsic) factors. This pathway is initiated by so-called contact activation, which is the activation of factor XII by contact with a negatively charged surface. In vivo, this could be subendothelial collagen exposed at the site of vascular injury. In vitro, kaolin, silica, celite, diatomaceous earth, or glass surfaces may be involved.
 - ♦ Once formed, surface-bound factor XIIa facilitates the binding of HMWK to the activating surface, probably by enzymatic cleavage of HMWK. Because HMWK circulates in association with PK and factor XI, factor XI and the three contact activation factors (PK, HMWK, and factor XIIa) become closely associated.
 - ♦ Factor XIIa activates PK to kallikrein, which enzymatically produces more kallikrein and more factor XIIa in a potent amplification pathway.
 - ♦ Factor XIIa cleaves factor XI, yielding factor XIa, which cleaves factor IX in the presence of fCa^{2+} to form factor IXa. Factor IXa then binds to the PL surface (in the presence of fCa^{2+}) to form the “tenase” or “Xase” complex with factor VIIIa (activated mostly by thrombin), which cleaves factor X to form factor Xa.
 - ♦ In vivo, the TF pathway is thought to initiate thrombin generation through activation of factors IX and X, and the surface-induced pathway is thought to propagate coagulation via thrombin feedback on factors XI, V and VIII. Surface activation plays a minor role in activating coagulation in vivo; patients with single contact factor deficiencies (e.g., factor XII, HMWK, or PK) do not have clinical bleeding disorders.

Fig. 5.2. continued

- **Common pathway:** This is the common continuation of the TF and surface-induced pathways, beginning with activation of factor X. Factor Xa complexes with factor Va (activated mostly by thrombin) and fCa^{2+} on a phospholipid surface to form the active *prothrombinase complex*, which results in the enzymatic conversion of prothrombin (factor II) to thrombin (factor IIa). Thrombin then activates platelets and cleaves its many substrates (not all shown), which include the following:
 - ♦ Fibrinogen: Fibrinopeptides A and B are cleaved from fibrinogen to form fibrin monomers, which polymerize into fibrin polymers (see Fig. 5.6).
 - ♦ Factor XIII: Proteolytic cleavage of factor XIII leads to activation. Factor XIIIa, in the presence of fCa^{2+} , cross-links fibrin and reinforces the secondary hemostatic plug.
 - ♦ Protein pro-cofactors (factors V and VIII): Proteolytic cleavage leads to cofactor activation and accelerated coagulation.
 - ♦ Factor XI: Thrombin activation provides positive feedback on the surface-induced and common pathways through factor XIa.
 - ♦ Protein C: APC inactivates factors Va and VIIIa, and promotes fibrinolysis via t-PA.
 - ♦ TAFI: TAFIa formed via thrombin and thrombomodulin inhibits fibrinolysis by cleaving plasminogen-binding sites from fibrin.

Fig. 5.3. Synthesis of vitamin K–dependent factors. Coagulation factors II (prothrombin), VII, IX, and X are synthesized primarily in hepatocytes. Vitamin K is required for these factors to be functional; that is, they are vitamin K dependent (as are the anticoagulants protein C, protein S, and protein Z).

- Vitamin K is ingested and also produced by intestinal bacteria. As a fat-soluble vitamin, it is absorbed with lipid that is digested by lipase and emulsified by the action of bile acids.
- In hepatocytes, vitamin K becomes reduced to its active form (reduced vitamin K). Reduced vitamin K is a cofactor for vitamin K–dependent carboxylase, the enzyme responsible for posttranslational gamma-carboxylation of glutamic acid residues in these coagulation factors. Carboxylation is needed so that the factors can bind fCa^{2+} , which induces conformational changes and enables binding to phospholipid membranes.
- Reduced vitamin K becomes oxidized to vitamin K epoxide during carboxylation, requiring enzymatic reduction before it can again function as a cofactor for vitamin K–dependent carboxylase.
- Carboxylated factors II, VII, IX, and X enter the blood, where they can be activated to participate in enzymatic reactions of the coagulation system. Vitamin K reductase is also known as NAD(P)H dehydrogenase (quinone).

Fig. 5.4. Antithrombin pathways. AT is the major physiologic anticoagulant in blood. It is produced primarily by hepatocytes and circulates in the blood, where it can inactivate thrombin (shown here) and the other coagulation enzymes. As shown schematically, AT activity is markedly enhanced in the presence of circulating unfractionated heparin or heparan sulfate on endothelial cells (1). Heparin or heparan sulfate bind to lysine sites of AT (2), inducing a conformational change in AT (note the larger triangular notches) that increases its affinity for thrombin. Thrombin, generated from the activation of prothrombin, then binds to the heparin-AT complex, forming a covalent 1:1 complex with AT (3). The thrombin-AT complexes (TAT) dissociate from the heparin or heparan sulfate and are cleared from circulation by hepatocytes. The heparin and heparan sulfate act as catalysts and are available (4) for forming more complexes. Anticoagulation by AT and heparin limits excessive clotting, but AT and heparin do not inhibit coagulation enzymes bound to fibrin or platelets. Therefore, localized and controlled coagulation can proceed where needed. LMWH molecules also induce conformational changes in AT that enable it to bind to and inhibit factor Xa, but not thrombin.

- Decreased plasma AT activity and concentration occur via consumption when intravascular coagulation is increased or after injection of exogenous heparin. Decreased concentrations may also occur from decreased hepatic production or from excessive loss due to protein-losing nephropathy or enteropathy. Decreased AT produces a prothrombotic state that heparin cannot counter well because heparin requires AT for most of its function.
- Increased plasma AT activity and concentration may occur with increased hepatic production of AT. This may occur secondary to the production of inflammatory cytokines.

Note: Nonphysiologic pathways are represented by *dotted arrows*.

Fig. 5.5. Schematic representation of the coagulation cascade as evaluated by in vitro screening coagulation tests. Fibrin formation is the end point for each test. PTT and ACT evaluate coagulation factors in the surface-induced pathway (factors XII, XI, IX, and VIII) and in the common pathway (factors X, V, II, and I). PT evaluates the short TF pathway (factor VII) and the common pathway. TT (and TT_{claus}) assesses only the conversion of fibrinogen (factor I) to fibrin with the addition of thrombin. Factor XIII is not assessed.

Fig. 5.6. Schematic representation of the formation of cross-linked fibrin from fibrinogen (*thin arrows*) and of the plasmin-mediated degradation of fibrinogen and fibrin to form FDPs (*thick arrows*).

- Fibrin formation: Fibrin is formed from fibrinogen, an elongated molecule that has a central E region and peripheral D regions (named for presence in the D and E fractions of FDPs eluted from an ion-exchange column). When thrombin is generated, it cleaves fibrinopeptides A and B from the E region of fibrinogen to form fibrin monomers. Unlike fibrinogen, fibrin monomers can polymerize (noncovalently) to form protofibrils of two or more strands (two are shown here). Thrombin also activates factor XIII to factor XIIIa, which cross-links adjacent D regions of different fibrin monomers to form stable cross-linked fibrin protofibrils. These can associate to form larger fibrin fibers (not shown) and a stable clot or thrombus.
- FDP formation: Plasmin cleaves fibrin and fibrinogen at specific sites to form fibrin fragments + fibrinogen fragments, collectively referred to as fibrin and fibrinogen degradation products (FDPs).
 - Fibrinogen: This is cleaved to form fragment X, and smaller fragments referred to as B β ₁₋₄₂ and A α polar appendages. Fragment X is further degraded to fragments D and Y, and fragment Y is degraded to fragments D and E.
 - Non-cross-linked fibrin: Note that the degradation of non-cross-linked fibrin is similar, differing only because of the removal of fibrinopeptides A and B from fibrinogen during the formation of fibrin. Fibrin monomers and non-cross-linked protofibrils are degraded to form fragment X' and smaller fragments B β ₁₅₋₄₂ and A α polar appendages. Fragment X' is further degraded to fragments D and Y', and fragment Y' is degraded to fragments D and E'.
 - Cross-linked fibrin: Plasmin-mediated degradation of cross-linked fibrin produces a different set of FDPs because of the covalent bonds formed by factor XIIIa between adjacent D regions. Initial degradation of cross-linked fibrin yields X-oligomers, high molecular weight compounds containing series of end-to-end X' segments. These may be further degraded to other FDPs, including fragments DD/E' (the major terminal breakdown product), Y'D/DY', and Y'Y'/DX'D.²²⁰
 - Antigenic similarities of FDPs produced from fibrinogen and fibrin make the current FDP assay nonspecific for fibrinogenolysis or fibrinolysis (with or without cross-linking). However, D-dimer assays detect a variety of cross-linked fibrin compounds that occur only with coagulation and fibrinolysis.

Fig. 5.7. Effects of vitamin K antagonism or deficiency on hepatocyte production of vitamin K–dependent coagulation factors (II, VII, IX, and X). *Circled numbers* in the figure denote the three following processes that result in production of defective factors II, VII, IX, and X:

1. Antagonism: Ingested anticoagulant rodenticides or other coumarins are absorbed in the intestine. In hepatocytes, they inhibit the enzymatic reduction of vitamin K epoxide back to reduced vitamin K, which is the form of vitamin K required for the vitamin K–dependent carboxylase enzyme to carboxylate the coagulation proteins. Failure to recycle vitamin K leads to decreased amounts of reduced vitamin K and increased amounts of vitamin K epoxide. Factors II, VII, IX, and X are still produced, however. They enter the blood and circulate in a permanently hypofunctional or nonfunctional state. These poorly carboxylated or noncarboxylated coagulation factors are known as PIVKA (proteins induced by vitamin K antagonism) and in people can be measured with specific antibodies. Plasma vitamin K epoxide concentrations increase (not shown). Vitamin K₁ administration overrides competitive inhibition of vitamin K reductase and enables functional factors to be produced.
2. Absence because of cholestasis (intrahepatic or posthepatic): Impaired bile flow reduces fat absorption because not enough bile acids are reaching the intestine to emulsify ingested fats properly. Consequently, fewer fat-soluble vitamins, including vitamin K, are absorbed, and a vitamin K deficiency may develop. The deficiency causes impaired carboxylation of vitamin K–dependent coagulation factors despite normal activity of reducing enzymes. Non-carboxylated factors are produced and enter the blood, where they are known as PIVKA (proteins induced by vitamin K absence). Amounts of vitamin K epoxide would not increase in this case.
3. Absence because of other causes: Anorexia (decreased dietary intake of vitamin K), decreased vitamin K production by intestinal bacteria (e.g., after antibiotics), intestinal malabsorptive disease, or exocrine pancreatic insufficiency can contribute to, or rarely cause, vitamin K deficiency.