



Published in final edited form as:

Toxicol Pathol. 2011 ; 39(1): 273–280. doi:10.1177/0192623310389474.

Current Understanding of Hemostasis

Andrew J. Gale, Ph.D.

Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California

Abstract

The goal of this review is to briefly summarize the two primary pathways of hemostasis, primary hemostasis and secondary hemostasis, as well as to summarize anticoagulant mechanisms and fibrinolysis. In addition, this review will discuss pathologies of hemostasis and the mechanisms of the various drugs that are available to impact these pathways to prevent either thrombosis or bleeding. While many of the main drugs that are used to treat disorders of hemostasis have been used for decades, greater understanding of hemostasis has led to development of various new drugs that have come onto the market recently or are close to coming onto the market. Thus, improved understanding of hemostasis continues to lead to benefits for patients.

Keywords

coagulation; platelets; hemophilia; thrombosis; hemostasis

Hemostasis is the physiological process that stops bleeding at the site of an injury while maintaining normal blood flow elsewhere in the circulation. Blood loss is stopped by formation of a hemostatic plug. The endothelium in blood vessels maintains an anticoagulant surface that serves to maintain blood in its fluid state, but if the blood vessel is damaged components of the subendothelial matrix are exposed to the blood. Several of these components activate the two main processes of hemostasis to initiate formation of a blood clot, composed primarily of platelets and fibrin. This process is tightly regulated such that it is activated within seconds of an injury but must remain localized to the site of injury.

There are two main components of hemostasis. Primary hemostasis refers to platelet aggregation and platelet plug formation. Platelets are activated in a multifaceted process (see below), and as a result they adhere to the site of injury and to each other, plugging the injury. Secondary hemostasis refers to the deposition of insoluble fibrin, which is generated by the proteolytic coagulation cascade. This insoluble fibrin forms a mesh that is incorporated into and around the platelet plug. This mesh serves to strengthen and stabilize the blood clot. These two processes happen simultaneously and are mechanistically intertwined. The fibrinolysis pathway also plays a significant role in hemostasis. Pathological thrombus formation, called thrombosis, or pathological bleeding can occur whenever this process is dis-regulated. The complexity of these systems has been increasingly appreciated in the last few decades.

Multiple anticoagulant mechanisms regulate and control these systems to maintain blood fluidity in the absence of injury and generate a clot that is proportional to the injury. The

Address correspondence to: Andrew J. Gale, Department of Molecular and Experimental Medicine, The Scripps Research Institute, MEM-286, 10550 N. Torrey Pines Rd, La Jolla, California 92037, Phone: (858) 784-2177, Fax (858) 784-2054, agale@scripps.edu.

Dr. Gale declares no relevant conflict of interest or financial disclosures.

proper balance between procoagulant systems and anticoagulant systems is critical for proper hemostasis and the avoidance of pathological bleeding or thrombosis.

Primary Hemostasis

Platelets are small anuclear cell fragments that bud off from megakaryocytes, specialized large polyploid blood cells that originate in the bone marrow (Schulze et al., 2005). Platelets are present at 150 to 400 million per milliliter of blood and circulate for about ten days (Zucker-Franklin, 2000). In a healthy blood vessel, and under normal blood flow, platelets do not adhere to surfaces or aggregate with each other. However, in the event of injury platelets are exposed to subendothelial matrix, and adhesion and activation of platelets begins (Figure 1).

Multiple receptors on the surface of platelets are involved in these adhesive interactions, and these receptors are targeted by multiple adhesive proteins. Detailed descriptions are available in these recent reviews (Jackson, 2007; Ruggeri et al., 2007; Varga-Szabo et al., 2008). The key for all of these receptors is that the adhesive interaction only takes place in the event of an injury to the blood vessel. This restriction is maintained in several different ways.

Receptor GPIb-IX-V binds to immobilized von Willebrand factor (VWF) specifically through an interaction between GPIb α and the A1 domain of VWF. VWF is a large multimeric protein secreted from endothelial cells and megakaryocytes that is always present in the soluble state in the plasma as well as in the immobilized state in subendothelial matrix (Ruggeri, 2007). However, soluble VWF in the circulation does not bind with high affinity to GPIb α (Yago et al., 2008). The high affinity interaction may be dependent upon high shear stress exerted by flowing blood on immobilized VWF, whether that VWF is immobilized on subendothelial matrix or other activated platelets (Siedlecki et al., 1996).

Receptor GPVI is constitutively active but its ligand is collagen, which is present in the subendothelial matrix and thus is only exposed to the blood in the event of injury. GPVI and GPIb-IX-V are critical for adhesion of platelets to subendothelial matrix at the site of injury and for their subsequent activation (Kehrel et al., 1998; Nieswandt et al., 2001).

Activation of platelets is critical for aggregation. In particular the integrins, α IIB β 3, α 2 β 1 and α v β 3 are normally present on the platelet surface in an inactive form, but platelet activation induces a conformational transition in these receptors that exposes ligand binding sites (Luo et al., 2006; Xiao et al., 2004). α IIB β 3 is arguably the most important of these receptors as it is present at the highest density on the platelet surface. In addition, α IIB β 3 binds to multiple ligands that promote platelet-platelet aggregation. These include fibrinogen, VWF, collagen, fibronectin and vitronectin (Varga-Szabo et al., 2008). α 2 β 1, α v β 3, α 5 β 1 and α 6 β 1 play smaller roles, binding primarily to collagen, vitronectin, fibronectin or laminin, respectively (Emsley et al., 2000; Lam et al., 1989; Sonnenberg et al., 1988; Varga-Szabo et al., 2008), though other ligands have also been identified for each of these. All of the integrins are maintained in an inactive state on quiescent platelets.

Feedback activation of nearby platelets surrounding a new site of injury is critical for further aggregation and propagation of the platelet plug. This activation is mainly mediated by agonists released by activated platelets themselves acting on G protein-coupled receptors. ADP is released from platelet dense granules and binds to receptors P2Y₁ and P2Y₁₂ (Mills, 1996). Thromboxane A₂ is synthesized de novo by activated platelets and binds to the thromboxane receptor primarily, and other prostanoid receptors to a lesser degree, locally on

platelets (Hanasaki et al., 1988). Serotonin is also secreted from dense granules and contributes to platelet activation.

Another critical mechanism of platelet activation that links secondary hemostasis to platelet function is activation by thrombin. Thrombin is the terminal serine protease of the coagulation cascade (see below). Thrombin cleaves 2 protease activated receptors (PARs) on human platelets, PAR1 and PAR4. These are also G protein-coupled receptors, and cleavage by thrombin exposes a new N-terminus that serves as a tethered ligand to activate the receptor (Kahn et al., 1998; Vu et al., 1991). All of these receptors initiate cell-signaling pathways when they are ligated, which result in platelet granule secretion, integrin activation and platelet cytoskeleton remodeling (Brass, 2000).

Thus, in summary, platelet adhesion is initiated by GPIIb binding to immobilized VWF and GPVI binding to collagen, which is exposed to the blood due to endothelial injury. These platelets and other local platelets are then activated, and adhesion and aggregation is strengthened and expanded via platelet-platelet connections between α IIB β 3 bound to fibrinogen, VWF, fibronectin or vitronectin as well as between α v β 3 bound to vitronectin or thrombospondin, with α 5 β 1-fibronectin and α 6 β 1-laminin interactions perhaps also playing a role. In addition, adherence to subendothelial collagen is strengthened via interaction of integrin α 2 β 1 and collagen. This platelet plug is also stabilized by deposition of insoluble fibrin generated by the coagulation cascade (see below).

Secondary Hemostasis

Secondary hemostasis consists of the cascade of coagulation serine proteases that culminates in cleavage of soluble fibrinogen by thrombin (Figure 2). Thrombin cleavage generates insoluble fibrin that forms a crosslinked fibrin mesh at the site of an injury. Fibrin generation occurs simultaneously to platelet aggregation (Falati et al., 2002;Furie, 2009). In intact and healthy blood vessels this cascade is not activated and several anticoagulant mechanisms prevent its activation. These include the presence of thrombomodulin and heparan sulfate proteoglycans on vascular endothelium. Thrombomodulin is a cofactor for thrombin that converts it from a procoagulant to an anticoagulant by stimulating activation of the anticoagulant serine protease protein C (Esmon et al., 1981). Heparan sulfate proteoglycans stimulate the activation of the serine protease inhibitor (or serpin) antithrombin, which inactivates thrombin and factor Xa (Shimada et al., 1991).

When the vascular system is injured, blood is exposed to extravascular tissues, which are rich in tissue factor (TF), a cofactor for the serine protease factor VIIa (Kirchhofer et al., 1996). The complex of TF and factor VIIa activates factor X and factor IX. This activation pathway is historically termed the extrinsic pathway of coagulation. Factor IXa also activates factor X, in the presence of its cofactor factor VIIIa. Factor Xa, also in the presence of its cofactor factor Va, then activates prothrombin to generate thrombin (Dahlback, 2000).

Thrombin is the central serine protease in the coagulation cascade, and it executes several critical reactions (Lane et al., 2005). Thrombin critically cleaves fibrinogen to generate insoluble fibrin. Thrombin activates platelets via cleavage of PAR1 and PAR4 (Kahn et al., 1998). Thrombin is also responsible for positive feedback activation of coagulation that is critical for clot propagation. Thrombin activates factor XI, which then activates factor IX and thrombin activates cofactors VIII and V (Lane et al., 2005). This has historically been called the intrinsic pathway of coagulation, but it is more appropriate to think of it as a positive feedback loop (Bouma et al., 1998).

The updated cell-based model of hemostasis focuses on the important fact that these reactions are controlled by their localization on different cellular surfaces. Coagulation is

initiated by the cofactor TF (the extrinsic pathway), which is a transmembrane protein present on fibroblasts and other extravascular tissues. The factor Xa generated here forms prothrombinase complex on these surfaces sufficient to generate only a small amount of thrombin. Then amplification and propagation of coagulation via the positive feedback loop occurs on the surface of platelets, which are activated near the site of injury by that trace thrombin and by adherence to extracellular matrix. Thus, the active coagulation complexes of this positive feedback loop form on the surface of activated platelets (Hoffman et al., 2001).

Ultimately thrombin also plays an important role in down regulation of the coagulation cascade by binding to thrombomodulin on endothelial cells and then activating protein C (APC) (Esmon et al., 1981). The activated protein C anticoagulant system is important for the down regulation of the coagulation cascade. APC cleaves and inactivates the procoagulant cofactors VIIIa and Va (Fulcher et al., 1984;Guinto et al., 1984). This reaction also requires a cofactor, protein S; in addition, factor V provides anticoagulant function as a cofactor for APC/protein S in the inactivation of factor VIIIa and factor Va (Cramer et al., 2010;Shen et al., 1994;Walker, 1980). These complexes between proteases and cofactors (procoagulant and anticoagulant) form on negatively charged membrane surfaces that are provided by activated platelets (Mann et al., 1990). This localization of the coagulation cascade reactions is critical to restrict coagulation to the site of injury.

The coagulation cascade is also down-regulated by inactivation of all the serine proteases by serine protease inhibitors. Most of these inhibitors are in the serpin family of inhibitors (Rau et al., 2007). Antithrombin is arguably the most important of these (Egeberg, 1965). Antithrombin inhibits thrombin and factor Xa, as well as factor IXa and factor XIa in the presence of heparin or heparan sulfate (Quinsey et al., 2004). Other serpins that play roles in coagulation include heparin cofactor II (thrombin inhibitor), protein Z-dependent protease inhibitor (factor Xa inhibitor), protein C inhibitor (APC inhibitor) and C1-inhibitor (factor XIa inhibitor) (Han et al., 2000;Marlar et al., 1980;Suzuki et al., 1983;Tollefsen et al., 1982;Wuillemin et al., 1995). Two non-serpin inhibitors, tissue factor pathway inhibitor and alpha-2-macroglobulin, also play a significant role, inhibiting factor Xa and thrombin, respectively (Broze, Jr. et al., 1988; James et al., 1967).

Fibrinolysis

The role of the fibrinolytic system is to dissolve blood clots during the process of wound healing and to prevent blood clots in healthy blood vessels. The fibrinolytic system is composed primarily of three serine proteases that are present as zymogens (i.e., proenzymes) in the blood. Plasmin cleaves and breaks down fibrin. Plasmin is generated from the zymogen plasminogen by the proteases tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA). TPA and plasminogen come together on the surface of a fibrin clot, to which they both bind. TPA then activates plasminogen, which subsequently cleaves fibrin. UPA activates plasminogen in the presence of the uPA receptor, which is found on various cell types (Lijnen et al., 2000). All three of these serine proteases are down-regulated by serpins that are present in blood. Alpha-2-antiplasmin inhibits plasmin, and plasminogen activator inhibitors 1 and 2 inhibit tPA and uPA (Rau et al., 2007).

Pathology of Hemostasis

Proper hemostasis is a function of balance between procoagulant systems (platelets, coagulation cascade) and anticoagulant systems (APC/protein S, fibrinolysis, serpins). If hemostasis is out of balance due to a defect in one of these systems, then either thrombosis (too much clotting) or bleeding (not enough clotting) may be the result.

Thrombosis

Arterial thrombi are composed largely of aggregated platelets, whereas venous thrombi are composed more of fibrin with red blood cells enmeshed. The composition of these different thrombi is dictated by the different conditions in the arterial circulation and the venous circulation. One important aspect of this is blood flow, with higher flow rates and therefore higher shear forces in the arterial circulation (Tangelder et al., 1988). Classically, arterial thrombosis and venous thrombosis are thought to have different risk factors. However, recent studies have suggested that some of the classic risk factors for arterial thromboses, such as obesity and high cholesterol, are also risk factors for venous thrombosis (Franchini et al., 2008). The classic risk factors for venous thrombosis cause a hypercoagulable state and result in an increased tendency for activation of the coagulation cascade. These include acquired risk factors such as cancer, surgery, immobilization, fractures and pregnancy. Genetic risk factors include multiple variants in the coagulation cascade. The most common are factor V_{Leiden} and prothrombin G20210A. Others are protein C or protein S heterozygosity and mutations in antithrombin (Bauer, 2000).

Arterial thrombosis is generally treated with drugs that inhibit platelet aggregation. Acetylsalicylic acid (aspirin) is a cyclo-oxygenase (COX) inhibitor and irreversibly inhibits COX-1 in the thromboxane A₂ synthesis pathway (Table 1)(Burch et al., 1978). Clopidogrel (trade name Plavix) blocks adenosine diphosphate (ADP) activation of platelets by inhibiting the ADP receptor P2Y₁₂ (Mills et al., 1992). Prasugrel (trade name Effient) is a new P2Y₁₂ inhibitor that was recently approved in the United States for certain indications (Sugidachi et al., 2001). Several new P2Y₁₂ receptor antagonists are in clinical trials, but none of them has been approved as of yet. Unlike clopidogrel, some of these are reversible inhibitors and thus might only temporarily inhibit platelet aggregation (Giossi et al., 2010). These might be more appropriate for acute short-term treatment rather than for long-term prophylaxis. There are also several other drugs that inhibit platelet aggregation whose use is less widespread. There are three α IIb β 3 integrin inhibitors, abciximab, eptifibatid and tirofiban. These are all intravenous (IV) drugs that are used primarily to treat or prevent acute coronary events in hospital (Cohen, 2009). Dipyridamole (Persantine) is an oral drug that inhibits adenosine reuptake and thromboxane synthesis and is used for secondary prevention of stroke and transient ischaemic attack (Weber et al., 2009).

Venous thrombosis is treated with drugs that inhibit the coagulation cascade. Historically this has included unfractionated heparin (UFH), which stimulates inhibition of coagulation serine proteases by antithrombin (Damus et al., 1973). UFH is only bioavailable via IV injection, which limits its utility to hospital care. Long-term prophylaxis has typically been maintained with one of several coumarine derivatives, which are vitamin K antagonists (e.g. warfarin, [trade name Coumadin], acenocoumarol [trade name Sintrom]). Vitamin K antagonists inhibit post-translational processing of the vitamin K-dependent coagulation serine proteases and therefore down-regulate the coagulation cascade overall (Furie et al., 1990).

More recently alternatives to these two classes of drugs have come onto the market, and many more are advancing through clinical trials. The first of these was low molecular weight heparin (LMWH), which is UFH that is broken down into smaller fragments either chemically or enzymatically (Weitz, 1997). Multiple versions of LMWH are on the market and they all function similarly but they are not identical. The advantages of these versus UFH are that they are bio-available via subcutaneous injection and proper dosing is much less variable. The next generation of LMWH was the synthetic LMWH mimic, fondaparinux. Fondaparinux is a sulfated pentasaccharide that stimulates antithrombin inactivation of factor Xa but is too small to stimulate inactivation of thrombin (Bauer, 2001).

Idraparinux is very similar to fondaparinux, but it has a longer half-life. However, idraparinux is not yet approved for use, and the longer half-life is actually a concern since there is no reversal agent. A variant of idraparinux is under investigation. This variant contains a biotin moiety, which binds with high affinity to the egg white protein avidin. Avidin could then serve as a reversal agent (Harenberg, 2009).

A new class of anticoagulant drugs is small molecules that directly inhibit factor Xa or thrombin. Many of these are being developed as oral drugs, which could be a significant advantage over LMWH or fondaparinux. Rivaroxaban is the only direct factor Xa inhibitor that is currently approved, but it is still not yet approved in the United States. Apixaban, betrixaban and edoxiban are similar drugs that are still in clinical trials. Initially these are in trials for relatively short-term prophylaxis during/after orthopedic surgery, but the hope is that they would ultimately prove safe for long-term prophylaxis.

Direct thrombin inhibitors include argatroban, which is approved in the US. However, argatroban is administered via IV. Hirudin, another direct thrombin inhibitor, is a polypeptide produced in the saliva of leeches. Several recombinant hirudin derivatives are approved for IV administration. These hirudin derivatives are primarily used during acute percutaneous interventions in patients in which heparin is contra-indicated. Dabigatran is the only oral direct thrombin inhibitor that is currently approved, but it is not yet approved in the United States. If oral direct thrombin or direct factor Xa inhibitors ultimately prove safe for long-term prophylaxis, they could potentially replace warfarin, which is less than ideal because of variability in dosing that requires regular monitoring to avoid over or under-dosing, which increases risk for bleeding and thrombosis (Laux et al., 2009).

Bleeding

The main bleeding disorders are genetically inherited. Hemophilia results from defects in secondary hemostasis. Hemophilia A is due to deficiency of factor VIII, and hemophilia B is due to deficiency of factor IX. Activated factor VIII and activated factor IX together form the intrinsic factor Xase complex on activated membrane surfaces that is critical in the positive feedback loop of blood coagulation (Dahlback, 2000). Therefore, deficiency in either of these proteins causes a very similar bleeding phenotype characterized by excess bruising, spontaneous bleeding into joints, muscles, internal organs and the brain. Factor VIII and factor IX are both X-linked genes. Thus hemophilia is primarily expressed in males, with hemophilia A present in about 1 in 5000 males and hemophilia B present in about 1 in 20,000 males. However, multiple mutations in either factor VIII or factor IX have been identified, and not all of them cause complete loss of protein or protein function. In fact, almost half of hemophilia A sufferers have *de novo* mutations that were not inherited from their parents. Depending on the mutation, hemophilia can be severe (<1% function), moderate (1–5%) or mild (5–20%) (Mannucci et al., 2001).

Hemophilia A and hemophilia B are treated mainly by infusion of recombinant factor VIII or factor IX, respectively. Before factor VIII and factor IX were available for infusion, hemophiliacs had a greatly reduced life expectancy and quality of life. In the 1960's and 1970's partially pure and then highly purified factor VIII or IX from human plasma became the standard therapy. However, the advent of HIV virus in the early 1980's resulted in a majority of hemophiliacs getting infected from factor preparations. As a result many hemophiliacs died, and many who did survive are infected with HIV. Virus inactivation procedures and restrictions on blood donations have now made plasma-derived protein preparations very safe (Mannucci, 2008). However, recombinant factor VIII and factor IX are ever more widely used out of a desire for even greater safety. This desire has largely led the development of improved 2nd and 3rd generation recombinant factor VIII products. 2nd

generation factor VIII is formulated without any animal- or human-derived proteins in the final formulation, but it is still generated in tissue culture medium containing animal products. These products include Kogenate FS, Helixate FS and Refacto. Recently, 3rd generation recombinant factor VIII has come on to the market. These products have no exposure to human or animal products during tissue culture production or during purification and formulation (Pipe, 2008). The third generation factor VIII's are Advate and Xyntha.

Von Willebrand disease (VWD) is a bleeding disorder caused by deficiency or defect in von Willebrand factor (VWF). VWF is involved in platelet aggregation and is also a carrier for factor VIII. Thus deficiency of VWF causes defects in platelet aggregation but also causes a deficiency of factor VIII (Ruggeri, 2007; Terraube et al., 2010). VWD can result from multiple different mutations in VWF. These different mutations cause various different forms of VWD. These have been grouped into three overall categories. Type 1 is a partial quantitative defect, while type 3 results from a complete absence of VWF. In type 2 VWD, a normal amount of VWF is present but it has functional defects. Type 2 VWD is broken down into several subcategories (Federici, 2008). VWF is an autosomal gene, so the disease is present equally in men and women. The severity of the different types of VWD varies, and various therapies are available and preferred for different forms of the disease.

The main drug used for treatment of type 1 (mild) and most type 2 VWD is desmopressin (DDAVP), which is a vasopressin analogue that stimulates release of VWF from endothelial cells and therefore temporarily increases the concentration of VWF in the blood. Type 3 VWD is more severe, and it is treated by infusion with pasteurized plasma-derived pure VWF/factor VIII concentrate (Humate-P). Both of these drugs can also be supplemented with the antifibrinolytic tranexamic acid. A significant risk for women with VWD is menorrhagia (abnormally heavy and prolonged menstrual period). This can often be treated with tranexamic acid or vasopressin, but another alternative is combined oral contraceptives to block menses (Federici, 2008; Rodeghiero et al., 2009).

There are several genetic platelet disorders that derive from defects in platelet receptors. Bernard-Soulier syndrome is due to a deficiency of GPIb-IX-V (Hagen et al., 1980). As a consequence, these individuals are defective in platelet aggregation because their platelets do not bind VWF. Bernard-Soulier syndrome is also characterized by abnormally large platelets and thrombocytopenia (Bennett, 2000). Glanzmann thrombasthenia is a deficiency of α IIb β 3 (Phillips et al., 1977). Since α IIb β 3 is also critical for platelet aggregation; and since it binds to fibrinogen, collagen, VWF, fibronectin and vitronectin; these individuals are also defective in platelet aggregation (Bennett, 2000). However, they have normal platelet numbers. The most common therapy for these disorders is transfusion of fresh frozen platelets during bleeding episodes or prophylactically during surgery. Deficiencies in collagen receptors and disorders in platelet secretion also exist. These are rare and typically result in mild bleeding. Treatment is usually transfusion of platelets (Bennett, 2000).

Hemostasis has now been widely studied for more than a century. In that time we have generated a very detailed picture of the molecular and cellular events that play roles in normal and pathological hemostasis. But there are still many interesting questions that remain to be answered. This research has also led to the development of numerous drugs that impact many different mechanisms of thrombosis or bleeding. Moving forward, drug development for treatment of hemostatic disorders is still a significant area of interest in the biotechnology and pharmaceutical industries. Therefore, the prospect for continued improvements in patient health and quality of life is great.

Acknowledgments

Dr. Gale was supported by NIH grant HL082588.

Abbreviations

VWF	von Willebrand factor
VWD	von Willebrand disease
TF	tissue factor

References

- Bauer, KA. Hypercoagulable States. In: Hoffman, R.; Benz, EJ.; Shattil, SJ.; Furie, B.; Silberstein, LE.; McGlave, P., editors. *Hematology Basic Principles and Practice*. 3. Churchill Livingstone; New York: 2000. p. 2009-2039.
- Bauer KA. Fondaparinux sodium: a selective inhibitor of factor Xa. *Am J Health Syst Pharm*. 2001; 58(Suppl 2):S14-S17. [PubMed: 11715834]
- Bennett, JS. Hereditary disorders of platelet function. In: Hoffman, R.; Benz, EJ.; Shattil, SJ.; Furie, B.; Silberstein, LE.; McGlave, P., editors. *Hematology Basic Principles and Practice*. 3. Churchill Livingstone; New York: 2000. p. 2154-2172.
- Bouma BN, von dem Borne PA, Meijers JC. Factor XI and protection of the fibrin clot against lysis--a role for the intrinsic pathway of coagulation in fibrinolysis. *Thromb Haemost*. 1998; 80:24-27. [PubMed: 9684779]
- Brass, LF. The molecular basis for platelet activation. In: Hoffman, R.; Benz, EJ.; Shattil, SJ.; Furie, B.; Cohen, HJ.; Silberstein, LE.; McGlave, P., editors. *Hematology Basic Principles and Practice*. 3. Churchill Livingstone; New York: 2000. p. 1753-1770.
- Broze GJ Jr, Warren LA, Novotny WF, Higuchi DA, Girard JJ, Miletich JP. The lipoprotein-associated coagulation inhibitor that inhibits the factor VII-tissue factor complex also inhibits factor Xa: insight into its possible mechanism of action. *Blood*. 1988; 71:335-343. [PubMed: 3422166]
- Burch JW, Stanford N, Majerus PW. Inhibition of platelet prostaglandin synthetase by oral aspirin. *J Clin Invest*. 1978; 61:314-319. [PubMed: 413839]
- Cohen M. Antiplatelet therapy in percutaneous coronary intervention: a critical review of the 2007 AHA/ACC/SCAI guidelines and beyond. *Catheter Cardiovasc Interv*. 2009; 74:579-597. [PubMed: 19472347]
- Cramer TJ, Griffin JH, Gale AJ. Factor V Is an Anticoagulant Cofactor for Activated Protein C during Inactivation of Factor Va. *Pathophysiol Haemost Thromb*. 2010; 37:17-23. [PubMed: 20501981]
- Dahlback B. Blood coagulation. *Lancet*. 2000; 355:1627-1632. [PubMed: 10821379]
- Damus PS, Hicks M, Rosenberg RD. Anticoagulant action of heparin. *Nature*. 1973; 246:355-357. [PubMed: 4586320]
- Egeberg O. Inherited antithrombin deficiency causing thrombophilia. *Thromb Diath Haemorrh*. 1965; 13:516-530. [PubMed: 14347873]
- Emsley J, Knight CG, Farndale RW, Barnes MJ, Liddington RC. Structural basis of collagen recognition by integrin alpha2beta1. *Cell*. 2000; 101:47-56. [PubMed: 10778855]
- Esmon CT, Owen WG. Identification of an endothelial cell cofactor for thrombin-catalyzed activation of protein C. *Proc Natl Acad Sci USA*. 1981; 78:2249-2252. [PubMed: 7017729]
- Falati S, Gross P, Merrill-Skoloff G, Furie BC, Furie B. Real-time in vivo imaging of platelets, tissue factor and fibrin during arterial thrombus formation in the mouse. *Nat Med*. 2002; 8:1175-1181. [PubMed: 12244306]
- Federici AB. Prophylaxis of bleeding episodes in patients with von Willebrand's disease. *Blood Transfus*. 2008; 6(Suppl 2):s26-s32. [PubMed: 19105507]
- Franchini M, Mannucci PM. Venous and arterial thrombosis: different sides of the same coin? *Eur J Intern Med*. 2008; 19:476-481. [PubMed: 19013373]

- Fulcher CA, Gardiner JE, Griffin JH, Zimmerman TS. Proteolytic inactivation of activated human factor VIII procoagulant protein by activated protein C and its analogy to factor V. *Blood*. 1984; 63:486–489. [PubMed: 6419799]
- Furie B. Pathogenesis of thrombosis. *Hematology Am Soc Hematol Educ Program*. 2009;255–258. [PubMed: 20008207]
- Furie B, Furie BC. Molecular basis of vitamin K-dependent gamma-carboxylation. *Blood*. 1990; 75:1753–1762. [PubMed: 2184900]
- Giossi A, Pezzini A, Del ZE, Volonghi I, Costa P, Ferrari D, Padovani A. Advances in antiplatelet therapy for stroke prevention: the new P2Y12 antagonists. *Curr Drug Targets*. 2010; 11:380–391. [PubMed: 20210760]
- Guinto ER, Esmon CT. Loss of prothrombin and of factor Xa-factor Va interactions upon inactivation of factor Va by activated protein C. *J Biol Chem*. 1984; 259:13986–13992. [PubMed: 6438088]
- Hagen I, Nurden A, Bjerrum OJ, Solum NO, Caen J. Immunochemical evidence for protein abnormalities in platelets from patients with Glanzmann's thrombasthenia and Bernard-Soulier syndrome. *J Clin Invest*. 1980; 65:722–731. [PubMed: 7354135]
- Han X, Fiehler R, Broze GJ Jr. Characterization of the protein Z-dependent protease inhibitor. *Blood*. 2000; 96:3049–3055. [PubMed: 11049983]
- Hanasaki K, Arita H. Characterization of thromboxane A2/prostaglandin H2 (TXA2/PGH2) receptors of rat platelets and their interaction with TXA2/PGH2 receptor antagonists. *Biochem Pharmacol*. 1988; 37:3923–3929. [PubMed: 2973322]
- Harenberg J. Development of idraparinix and idrabiotaparinux for anticoagulant therapy. *Thromb Haemost*. 2009; 102:811–815. [PubMed: 19888513]
- Hoffman M, Monroe DM III. A cell-based model of hemostasis. *Thromb Haemost*. 2001; 85:958–965. [PubMed: 11434702]
- Jackson SP. The growing complexity of platelet aggregation. *Blood*. 2007; 109:5087–5095. [PubMed: 17311994]
- James K, Taylor FB Jr, Fudenberg HH. The effect of alpha-2-macroglobulin in human serum on trypsin, plasmin, and thrombin activities. *Biochim Biophys Acta*. 1967; 133:374–376. [PubMed: 4166088]
- Kahn ML, Zheng YW, Huang W, Bigornia V, Zeng D, Moff S, Farese RV Jr, Tam C, Coughlin SR. A dual thrombin receptor system for platelet activation. *Nature*. 1998; 394:690–694. [PubMed: 9716134]
- Kehrel B, Wierwille S, Clemetson KJ, Anders O, Steiner M, Knight CG, Farndale RW, Okuma M, Barnes MJ. Glycoprotein VI is a major collagen receptor for platelet activation: it recognizes the platelet-activating quaternary structure of collagen, whereas CD36, glycoprotein IIb/IIIa, and von Willebrand factor do not. *lood*. 1998; 91:491–499.
- Kirchhofer D, Nemerson Y. Initiation of blood coagulation: the tissue factor/factor VIIa complex. *Curr Opin Biotechnol*. 1996; 7:386–391. [PubMed: 8768895]
- Lam SC, Plow EF, D'Souza SE, Cheresh DA, Frelinger AL III, Ginsberg MH. Isolation and characterization of a platelet membrane protein related to the vitronectin receptor. *J Biol Chem*. 1989; 64:3742–3749. [PubMed: 2465293]
- Lane DA, Philippou H, Huntington JA. Directing thrombin. *Blood*. 2005; 106:2605–2612. [PubMed: 15994286]
- Laux V, Perzborn E, Heitmeier S, von DG, Dittrich-Wengenroth E, Buchmuller A, Gerdes C, Misselwitz F. Direct inhibitors of coagulation proteins - the end of the heparin and low-molecular-weight heparin era for anticoagulant therapy? *Thromb Haemost*. 2009; 102:892–899. [PubMed: 19888525]
- Lijnen, HR.; Collen, D. Molecular and cellular basis of fibrinolysis. In: Hoffman, R.; Benz, EJ.; Shattil, SJ.; Furie, B.; Silberstein, LE.; McGlave, P., editors. *Hematology Basic Principles and Practice*. 3. Churchill Livingstone; New York: 2000. p. 1804-1814.
- Luo BH, Springer TA. Integrin structures and conformational signaling. *Curr Opin Cell Biol*. 2006; 18:579–586. [PubMed: 16904883]
- Mann KG, Nesheim ME, Church WR, Haley P, Krishnaswamy S. Surface-dependent reactions of the vitamin K-dependent enzyme complexes. *Blood*. 1990; 76:1–16. [PubMed: 2194585]

- Mannucci PM. Back to the future: a recent history of haemophilia treatment. *Haemophilia*. 2008; 14(Suppl 3):10–18. [PubMed: 18510516]
- Mannucci PM, Tuddenham EG. The hemophilias--from royal genes to gene therapy. *N Engl J Med*. 2001; 344:1773–1779. [PubMed: 11396445]
- Marlar RA, Griffin JH. Deficiency of protein C inhibitor in combined factor V/VIII deficiency disease. *J Clin Invest*. 1980; 66:1186–1189. [PubMed: 6253526]
- Mills DC. ADP receptors on platelets. *Thromb Haemost*. 1996; 76:835–856. [PubMed: 8971999]
- Mills DC, Puri R, Hu CJ, Minniti C, Grana G, Freedman MD, Colman RF, Colman RW. Clopidogrel inhibits the binding of ADP analogues to the receptor mediating inhibition of platelet adenylate cyclase. *Arterioscler Thromb*. 1992; 12:430–436. [PubMed: 1558834]
- Nieswandt B, Brakebusch C, Bergmeier W, Schulte V, Bouvard D, Mokhtari-Nejad R, Lindhout T, Heemskerk JW, Zirngibl H, Fassler R. Glycoprotein VI but not alpha2beta1 integrin is essential for platelet interaction with collagen. *EMBO J*. 2001; 20:2120–2130. [PubMed: 11331578]
- Phillips DR, Agin PP. Platelet membrane defects in Glanzmann's thrombasthenia. Evidence for decreased amounts of two major glycoproteins. *J Clin Invest*. 1977; 60:535–545. [PubMed: 70433]
- Pipe SW. Recombinant clotting factors. *Thromb Haemost*. 2008; 99:840–850. [PubMed: 18449413]
- Quinsey NS, Greedy AL, Bottomley SP, Whisstock JC, Pike RN. Antithrombin: in control of coagulation. *Int J Biochem Cell Biol*. 2004; 36:386–389. [PubMed: 14687916]
- Rau JC, Beaulieu LM, Huntington JA, Church FC. Serpins in thrombosis, hemostasis and fibrinolysis. *J Thromb Haemost*. 2007; 5(Suppl 1):102–115. [PubMed: 17635716]
- Rodeghiero F, Castaman G, Tosetto A. How I treat von Willebrand disease. *Blood*. 2009; 114:1158–1165. [PubMed: 19474451]
- Ruggeri ZM. The role of von Willebrand factor in thrombus formation. *Thromb Res*. 2007; 120(Suppl 1):S5–S9. [PubMed: 17493665]
- Ruggeri ZM, Mendolicchio GL. Adhesion mechanisms in platelet function. *Circ Res*. 2007; 100:1673–1685. [PubMed: 17585075]
- Schulze H, Shivdasani RA. Mechanisms of thrombopoiesis. *J Thromb Haemost*. 2005; 3:1717–1724. [PubMed: 16102038]
- Shen L, Dahlback B. Factor V and protein S as synergistic cofactors to activated protein C in degradation of factor VIIIa. *J Biol Chem*. 1994; 269:18735–18738. [PubMed: 8034625]
- Shimada K, Kobayashi M, Kimura S, Nishinaga M, Takeuchi K, Ozawa T. Anticoagulant heparin-like glycosaminoglycans on endothelial cell surface. *Jpn Circ J*. 1991; 55:1016–1021. [PubMed: 1744977]
- Siedlecki CA, Lestini BJ, Kottke-Marchant KK, Eppell SJ, Wilson DL, Marchant RE. Shear-dependent changes in the three-dimensional structure of human von Willebrand factor. *Blood*. 1996; 88:2939–2950. [PubMed: 8874190]
- Sonnenberg A, Modderman PW, Hogervorst F. Laminin receptor on platelets is the integrin VLA-6. *Nature*. 1988; 336:487–489. [PubMed: 2973567]
- Sugidachi A, Asai F, Yoneda K, Iwamura R, Ogawa T, Otsuguro K, Koike H. Antiplatelet action of R-99224, an active metabolite of a novel thienopyridine-type G(i)-linked P2T antagonist, CS-747. *Br J Pharmacol*. 2001; 132:47–54. [PubMed: 11156560]
- Suzuki K, Nishioka J, Hashimoto S. Protein C inhibitor: Purification from human plasma and characterization. *J Biol Chem*. 1983; 258:163–168. [PubMed: 6294098]
- Tangelder GJ, Slaaf DW, Arts T, Reneman RS. Wall shear rate in arterioles in vivo: least estimates from platelet velocity profiles. *Am J Physiol*. 1988; 254:H1059–H1064. [PubMed: 3381893]
- Terraube V, O'Donnell JS, Jenkins PV. Factor VIII and von Willebrand factor interaction: biological, clinical and therapeutic importance. *Haemophilia*. 2010; 16:3–13. [PubMed: 19473409]
- Tollefsen DM, Majerus DW, Blank MK. Heparin cofactor II. Purification and properties of a heparin-dependent inhibitor of thrombin in human plasma. *J Biol Chem*. 1982; 257:2162–2169. [PubMed: 6895893]
- Varga-Szabo D, Pleines I, Nieswandt B. Cell adhesion mechanisms in platelets. *Arterioscler Thromb Vasc Biol*. 2008; 28:403–412. [PubMed: 18174460]

- Vu TK, Hung DT, Wheaton VI, Coughlin SR. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell*. 1991; 64:1057–1068. [PubMed: 1672265]
- Walker FJ. Regulation of activated protein C by a new protein. A possible function for bovine protein S. *J Biol Chem*. 1980; 255:5521–5524. [PubMed: 6892911]
- Weber R, Weimar C, Diener HC. Medical prevention of stroke and stroke recurrence in patients with TIA and minor stroke. *Expert Opin Pharmacother*. 2009; 10:1883–1894. [PubMed: 19558342]
- Weitz JI. Low-molecular-weight heparins. *N Engl J Med*. 1997; 337:688–698. [PubMed: 9278467]
- Wuillemin WA, Minnema M, Meijers JC, Roem D, Eerenberg AJ, Nuijens JH, ten Cate H, Hack CE. Inactivation of factor XIa in human plasma assessed by measuring factor XIa-protease inhibitor complexes: major role for C1-inhibitor. *Blood*. 1995; 85:1517–1526. [PubMed: 7534133]
- Xiao T, Takagi J, Collier BS, Wang JH, Springer TA. Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. *Nature*. 2004; 432:59–67. [PubMed: 15378069]
- Yago T, Lou J, Wu T, Yang J, Miner JJ, Coburn L, Lopez JA, Cruz MA, Dong JF, McIntire LV, McEver RP, Zhu C. Platelet glycoprotein Ibalph forms catch bonds with human WT vWF but not with type 2B von Willebrand disease vWF. *J Clin Invest*. 2008; 118:3195–3207. [PubMed: 18725999]
- Zucker-Franklin, D. Megakaryocyte and platelet structure. In: Hoffman, R.; Benz, EJ.; Shattil, SJ.; Furie, B.; Silberstein, LE.; McGlave, P., editors. *Hematology Basic Principles and Practice*. 3. Churchill Livingstone; New York: 2000. p. 1730-1740.

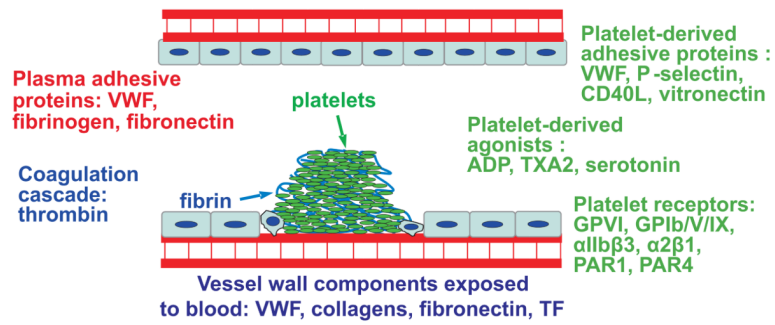


Figure 1.

Primary hemostasis; the platelet response. Platelet aggregation at the site of injury is mediated by platelet receptors, platelet-derived agonists, platelet-derived adhesive proteins and plasma-derived adhesive proteins. Fibrin deposition around the resulting platelet plug is generated by the coagulation cascade (Figure 2). ADP = adenosine diphosphate, TXA2 = thromboxane A2 and VWF = von Willebrand factor.

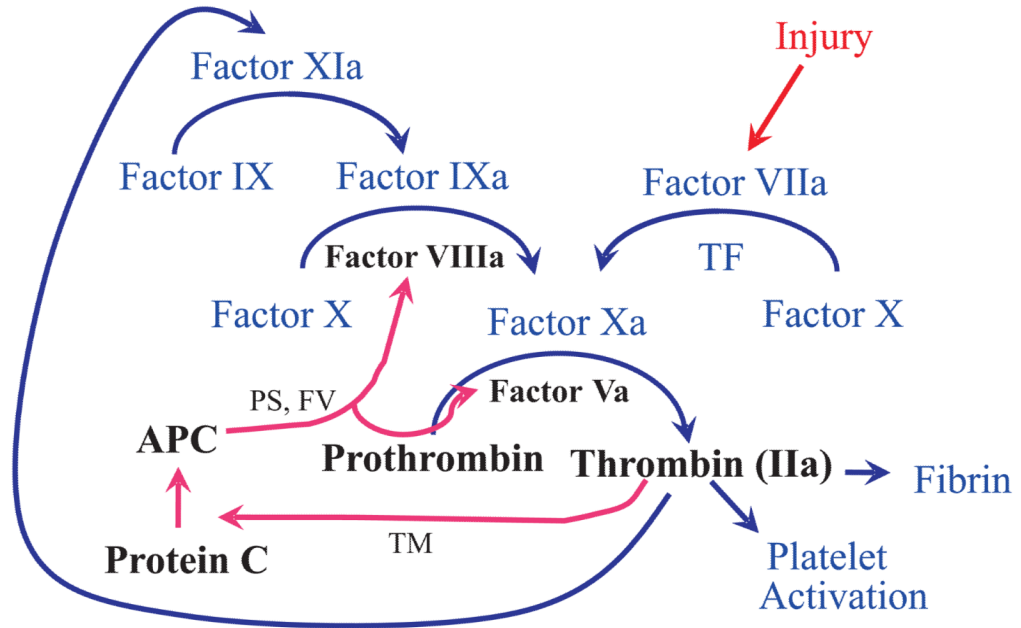


Figure 2.

Secondary hemostasis; the coagulation cascade. At the site of injury, tissue factor (TF) initiates the coagulation cascade that results in the formation of the serine protease thrombin. Thrombin performs multiple functions, including fibrin generation, platelet activation, positive feedback activation of the intrinsic pathway, and negative feedback activation of the activated protein C pathway. Procoagulant reactions are shown in blue, and anticoagulant reactions are shown in pink. APC = activated protein C, FV = factor V, PS = protein S and TM = thrombomodulin.

Table 1

Pharmaceutical agents currently used to treat hemostatic disorders

Drug	Trade name	Targeted molecule/system	Clinical use
acetylsalicylic acid	Aspirin	cyclo-oxygenase, TXA ₂ synthesis	block platelet aggregation, arterial thrombosis
clopidogrel	Plavix	ADP receptor P2Y ₁₂	block platelet activation, arterial thrombosis
prasugrel	Effient	ADP receptor P2Y ₁₂	block platelet activation, arterial thrombosis
abciximab	ReoPro	integrin αIIbβ3	block platelet aggregation, arterial thrombosis
eptifibatid	Integrilin	integrin αIIbβ3	block platelet aggregation, arterial thrombosis
tirofiban	Aggrastat	integrin αIIbβ3	block platelet aggregation, arterial thrombosis
dipyridamol	Persantine	adenosine reuptake	block platelet activation, arterial thrombosis
unfractionated heparin	Heparin	thrombin, factor Xa	blocks coagulation, venous thrombosis
low molecular weight heparin	several names, e.g. Lovenox	factor Xa	blocks coagulation, venous thrombosis
fondaparinux	Arixtra	factor Xa	blocks coagulation, venous thrombosis
rivaroxaban	Xarelto	factor Xa	blocks coagulation, venous thrombosis
argatroban	Argatroban	thrombin	prevent thrombosis during percutaneous coronary intervention (PCI)
dabigatran	Pradaxa	thrombin	prevent thrombosis during/after joint surgery
bivalirudin	Angiomax	thrombin	prevent thrombosis during PCI
lepirudin	Refludan	thrombin	prevent thrombosis in patients with heparin- induced thrombocytopenia
desirudin	Iprivask	thrombin	venous thrombosis during/after joint surgery
coumarin derivatives	several names, e.g. Coumadin	vitamin K antagonist	venous thrombosis, long-term prophylaxis
factor VIII	multiple names	replace factor VIII	hemophilia A
factor IX	multiple names	replace factor IX	hemophilia B
desmopressin	several names, e.g. DDAVP	von Willebrand factor	von Willebrand disease
tranexamic acid	Lysteda	fibrinolysis	von Willebrand disease
von Willebrand factor	Humate-P	von Willebrand factor	von Willebrand disease