# Chapter 9
# Physiological Haemostasis


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**Key Learning Points**

- The haemostatic response involves complex interactions between multiple proteins and cell types, and can be divided into primary and secondary haemostasis
- Primary haemostasis results in platelet plug formation and results from interactions between platelets and subendothelial adhesive proteins (von Willebrand factor and collagen)
- As part of the primary haemostatic response, platelets go through a sequence of events that involves initial platelet adhesion, resulting in intracellular signalling that triggers platelet shape change, activation with granule release, and finally aggregation.
- Secondary haemostasis results in sequential conversion of zymogens to active enzymes as part of the coagulation cascade, leading to the formation of a mesh-like network of cross-linked fibrin.
- The fibrinolytic system is responsible for the dissolution of thrombus composed of cross-linked fibrin, and plays a major role in helping maintain a patent vascular system

## 9.1 Introduction

Physiological haemostasis involves complex interactions between endothelial cells, platelets, and coagulation proteins, that result in a platelet plug and localised thrombus formation at the site of a break in vascular integrity. Numerous regulatory processes prevent widespread activation of coagulation, ensuring that blood remains fluid in the absence of vascular injury or other pathology. All components of the

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haemostatic process can be disturbed resulting in either a pro-thrombotic or bleeding tendency, and drugs that modify the haemostatic process are commonly used, particularly in patients with vascular disease. An understanding of normal haemostasis is therefore important for all clinicians that deal with this patient group.

## 9.2 Primary Haemostasis

Primary haemostasis is the initial response of the body to vascular injury, and involves interaction between platelets, adhesive proteins located in the subendothelial matrix (including collagen and von Willebrand factor), and circulating fibrinogen [1]. The end result of primary haemostasis is the formation of a stable platelet plug around which a fibrin network can then be built. This same process is responsible for the pathogenic thrombus formation in patients with arterial disease. Disorders of primary haemostasis tend to manifest as mucosal bleeding, including epistaxis, oral bleeding and menorrhagia, and often result in immediate difficulty with haemostasis in the post-operative setting.

### *9.2.1 Platelets*

Platelets are small fragments of megakaryocyte cytoplasm that, in the resting state, are small discoid structures. The normal range for circulating platelet count in adults is between 150 and 400 × $10^9$/L. Although anucleate, platelets are metabolically active, and interact with the local environment through the binding of surface glycoprotein receptors to specific ligands (for more detail on these interactions, see Chap. 10). Platelets go through a predictable cycle of response to vessel wall injury that involves initial platelet adhesion to the sub-endothelium, subsequent intracellular signalling that triggers platelet shape change and activation with granule release, and finally aggregation (Fig. 9.1) [2].

#### 9.2.1.1 Platelet Adhesion and von Willebrand Factor

Endothelial injury results in the exposure of circulating blood to the subendothelial matrix that is rich in a number of adhesive proteins. von Willebrand factor (vWF) is a large adhesive glycoprotein produced by endothelial cells and megakaryocytes that play a central role in initial platelet adhesion [3]. The mature vWF molecule consists of disulphide-linked multimers of high molecular weight of up to 20,000,000 daltons [4]. When secreted into the plasma, these high molecular weight (HMW) vWF multimers are digested into smaller forms by the metalloprotease ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13). These smaller soluble forms bind less readily to platelet receptors, reducing the

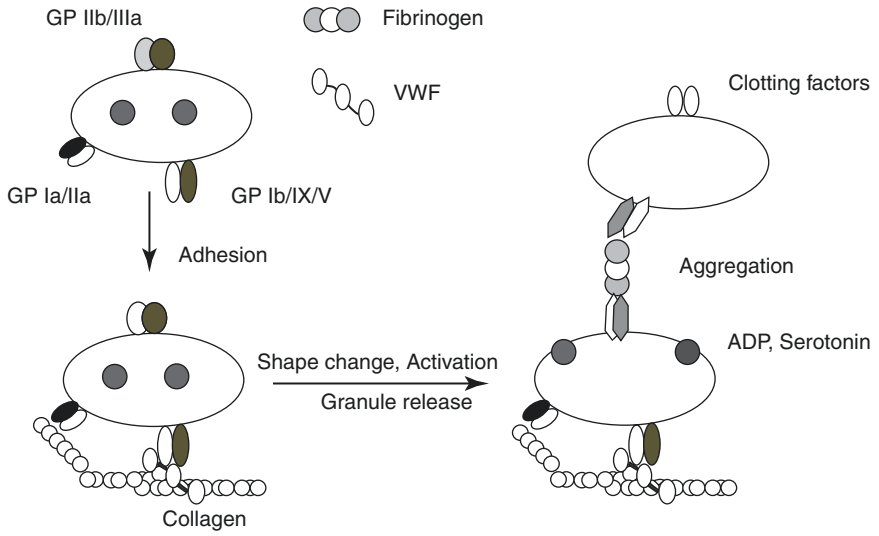


**Fig. 9.1** Mechanism of platelet aggregation

chance of spontaneous platelet aggregation. However, vWF secreted into the subendothelial space binds to other molecules such as collagen, resulting in a conformational change that exposes the binding site for platelet glycoprotein (GP) receptor Ib [4]. Subendothelial vWF is therefore "primed" to interact with circulating platelets in the event of endothelial injury. Other important adhesive proteins include collagen type 1 and type 4, fibronectin, thrombospondin, laminin and vitronectin.

Initial platelet adhesion, particularly in high shear conditions, involves interaction between vWF and the GPIb/IX/V complex located on the platelet surface. This complex consists of four trans-membrane subunits GPIba, GPIbb, GPIX and GPV, with the N-terminal globular domain of GPIba responsible for the interaction with the A1-domain of vWF [1]. Binding of vWF to GP Ib is often reversible, and in animal models platelets can be seen to initially slide or translocate along the subendothelial surface due to cyclical attachment and then dissociation of the GP Ib/IX/V complex to vWF [2]. However, finally through further platelet receptor ligand interactions the platelet is stabilized on the subendothelial surface. The platelet glycoprotein Ia/IIa receptor (integrin $\alpha_2\beta_1$) binds collagen, an interaction that appears to be more important in low-shear conditions [5]. Glycoprotein VI, a platelet surface receptor that belongs to the immunoglobin superfamily, also directly binds collagen and further activates the GPIa/IIa receptor via intracellular signaling [6]. Other $\beta_1$ integrins also bind their respective subendothelial ligands ($\alpha_6\beta_1$—laminin; $\alpha_5\beta_1$—fibronectin), and there is increasing evidence that early binding of vWF to the glycoprotein IIb/IIIa ($\alpha_{IIb}\beta_3$) receptor contributes to the initial adhesion process [2]. Finally there is evidence that formation of platelet membrane tethers, that consist of smooth cylinders of lipid membrane pulled from the platelet surface under the influence of hemodynamic drag forces, contribute to platelet adhesion in high shear conditions [7].

In addition to being involved in initial platelet adhesion as described above, VWF also binds circulating factor VIII [8]. This significantly prolongs the half-life of the latter molecule. As a result, patients with reduced VWF levels will also have reduced FVIII levels due to the acceleration in clearance of FVIII.

VWF has also been shown to be involved in the regulation of blood vessel formation, with lack of VWF leading to enhanced angiogenesis in some vascular beds [9]. As a result patients with von-Willebrand disease have been documented to have increased rates of angiodysplasia that may result in intractable gastro-intestinal bleeding.

#### 9.2.1.2 Platelet Activation and Shape Change

Following platelet adhesion, multiple pathways lead to platelet activation that results in platelet shape change, platelet granule release, and conformational change in the GP IIb/IIIa receptor that allows binding to fibrinogen and vWF, leading to platelet aggregation. Binding of vWF to the GP Ib receptor and collagen to the GP VI during the adhesion process triggers intracellular signaling via a pathway that involves activation of Src family kinases (Src), Syk and PI 3-kinase (PI3K). These events lead to the activation of phospholipase C-β (PLC), which hydrolyses membrane phospholipids to generate inositol (1,4,5) trisphosphate (IP3) [10]. The binding of IP3 to its receptors (IP3R) on the dense tubular system (DTS) then results in mobilisation of intra-platelet calcium stores, which has a number of consequences including;

1. *Thromboxane A2 (TXA2) generation*—the increase in intracellular calcium stimulates the production of arachadonic acid by PLC and phospholipase A2. Arachadonic acid is converted into TxA2 via the actions of the enzymes cyclooxygenase 1 (COX-1) and Tx synthase. TxA2 is released from the platelet and binds platelet receptors TPα and TPß. The effects of TxA2 in platelets are mediated primarily through TPα. Binding of TxA2 to this G-protein coupled receptor results in further PLC activation, leading to further intracellular calcium increase further reinforcing platelet activation [11]. Local diffusion of TxA2 also contributes to the recruitment to the site of injury and activation of further platelets. Aspirin or acetyl salicylic acid exerts its antiplatelet effect by blocking TXA2 synthesis, due to the irreversible acetylation of Serine-529 in COX-1. Because platelets are anucleate, no new COX can be generated, explaining why aspirin has a persistent functional effect that lasts the lifespan of the platelet (approximately 7 days).
2. *Granule release*—intracellular calcium mobilization also results in the release from the platelet of both the dense and alpha-granules. The dense granules contain high concentrations of the small molecules adenosine diphosphate (ADP) and serotonin, which further act to reinforce local platelet activation by binding to specific platelet surface membrane receptors upon release. ADP is a central player in sustained platelet activation. The receptors for ADP, the $P2Y_1$ and

$P2Y_{12}$ are seven transmembrane receptors that are coupled via heterotrimeric G-proteins to numerous intracellular effector molecules. $P2Y_1$ links to the G-protein Gq resulting in further activation of PLC and also protein kinase C activation. $P2Y_{12}$ is linked to the G-protein Gi that has an inhibitory effect on adenylate cyclase. ADP induced activation of the $P2Y_1$ receptor induces platelet shape change and rapid transient aggregation [12], whereas activation of the $P2Y_{12}$ receptor results in sustained irreversible aggregation [13]. The thienopyridine class of antiplatelet agents, ticlopidine, clopidogrel, prasugrel and ticagrelor all exert their antiplatelet effect by blocking the $P2Y_{12}$ receptor. The active metabolites of all agents have a free thiol moiety that forms a disulfide bridge with the extracellular cysteine residues Cys17 and Cys270 [14]. Released serotonin also binds to a G-protein coupled platelet surface receptor, the $5\text{-}HT_{2A}$ receptor. Binding is also associated with Gq-dependent activation of PLC, resulting in amplification of platelet activation, platelet shape change, and weak reversible platelet aggregation [15].

3. *Activation of the GP IIb/IIIa receptor*—in its resting state the GP IIb/IIIa receptor is unable to bind its ligands, namely fibrinogen and vWF. The above platelet signaling events through the activation of the small GTPase Rap1b and its interaction with a Rap1-GTP interacting adapter molecule (RIAM), lead to the binding of the proteins talin and kindlin to ß3 tail of GP IIb/IIIa receptor [16]. This leads to activation of the receptor and the resulting change in conformation allows the surface portion of the receptor to bind readily to fibrinogen and vWF. The binding of talin to the receptor tail also links it to the underlying actin cytoskeleton of the platelet, enhancing adhesive strength and platelet cohesion [17].
4. *Platelet shape change*—the normally discoid-shaped platelet with a smooth surface membrane undergoes dramatic shape change with stimulation, including extension of filopodia, and flattening or spreading on the subendothelial surface. The platelet cytoskeleton is primarily responsible for regulating the platelet's shape. Platelet activation leads to the rapid reorganization and polymerization of actin into filaments, resulting in the above conformational change [18].

Along with ADP, the serine protease thrombin plays an important role in sustaining platelet activation leading to irreversible platelet aggregation. Thrombin specific receptors, the protease-activated receptors (PARs), are located on the platelet surface. Two main PARs, PAR1 a high affinity receptor and PAR4, a low affinity receptor, are involved in thrombin mediated platelet activation [19]. Thrombin activates PARs by cleaving the N-terminal of the receptor, unmasking a hidden receptor-linked ligand. This ligand then interacts with the remainder of the receptor leading to G-protein coupled signaling that results in further platelet activation.

Finally platelet activation also results in the surface expression of a number of adhesion molecules, such as the glycoprotein P-selectin which is involved in interaction with both endothelial cells and also the recruitment of inflammatory cells to the area of injury, via binding of P-selectin to P-selectin glycoprotein ligand 1 (PSGL-1) located on the surface of leucocytes [20]. Platelets also secrete

chemokines such as RANTES/CCL5 and platelet factor 4 that also increase the local recruitment of inflammatory cells such as monocytes. This contributes to and can exacerbate the local inflammatory response that is often present in atherosclerotic plaque [21]. A more detailed outline of platelet activation is given in Chap. 10.

#### 9.2.1.3 Platelet Aggregation

As the final part of the primary haemostatic response, platelets recruited to the site of vascular injury and activated by the above soluble agonists then undergo irreversible aggregation. This is mediated via the concurrent binding of either fibrinogen or vWF to the activated GP IIb/IIIa receptors on separate platelets, leading to their cross-linking and the formation of a platelet aggregate. In low flow vascular beds, binding of fibrinogen to the GP IIb/IIIa receptor appears to be the main process involved in platelet aggregation, whereas the interaction between GP IIb/IIIa and vWF is more important for aggregation in high shear vascular beds and pathological arterial thrombosis [7].

## 9.3 Interactions Between Primary and Secondary Haemostasis

While the primary and secondary haemostatic processes are often considered separately, they are intrinsically linked. As described above, the coagulation protease thrombin plays a central role in the activation of platelets. The activated platelet in turn provides the surface upon which the reaction complexes of the coagulation cascade form. In addition, as part of platelet activation the content of the negatively charged phospholipid phosphatidylserine on the outer surface of the platelet membrane increases from almost 0% up to 12%, providing a binding site for the proteins of the coagulation cascade [22]. Release of clotting factors, such as factor V, from platelet alpha granules, and the expression of other as yet still poorly defined platelet receptors for coagulation factors on the platelet surface provide additional methods in which activation of the coagulation cascade is localised to the site of platelet activation and vascular injury [23].

## 9.4 Secondary Haemostasis

Secondary haemostasis describes the process whereby exposure of tissue factor to the bloodstream leads to a series of enzymatic reactions that result in a sufficient burst of thrombin production to convert soluble fibrinogen into a stable network. This process is mediated by the formation of a series of reaction complexes, each consisting of an active enzyme and a co-factor, in which the presence of the latter results in an order of magnitude increase in the efficiency of the enzyme to bind to

and convert its target substrate, itself a pro-enzyme or zymogen, to its active form. Defects of secondary haemostasis, as typified by factor VIII deficiency or haemophilia A, may result in muscle, joint, intracerebral and soft tissue bleeding, and delayed bleeding post surgical or traumatic haemostatic challenge.

The coagulation factors involved in secondary haemostasis belong to the class of proteins known as serine proteases, so called because they have a serine residue which, along with histidine and aspartic acid, forms a catalytic triad at the centre of the active site of the enzyme [23]. Most of the reactions of secondary haemostasis take place on a phospholipid membrane surface, which is normally the surface of an activated platelet. Binding of the coagulation proteins to the phospholipid membrane surface requires the presence of calcium, and agents that chelate calcium such as EDTA or citrate can therefore be utilised to prevent activation of the coagulation cascade after blood collection.

The coagulation factors have a modular structure, and different factors share similar structural features. The coagulation factors II, VII, IX, X along with the natural inhibitors of coagulation, protein C and protein S, all undergo post-translational gamma-carboxylation of glutamate residues located at the amino-terminus. This modification is necessary for the efficient binding of these proteins to phospholipid surfaces. The carboxylation process is dependant on the presence of vitamin K, which is a co-factor for this process. Vitamin K deficiency or Vitamin K antagonists, such as warfarin that prevent the conversion of vitamin K to its reduced form by blocking the activity of the enzyme vitamin K epoxide-reductase, leading to a reduction in the activity of the coagulation factors, resulting in an anticoagulant effect.

## 9.5 The Coagulation Cascade

Early observations noted that exposure of blood or plasma to surfaces such as glass would also precipitate clot formation without the addition of further material (intrinsic activation of coagulation), and that this process could be accelerated by the addition of exogenous biological material such as macerated brain extract (extrinsic activation of coagulation). These observations led to the concept of “extrinsic” and “intrinsic” pathways of coagulation, and over time the coagulation factors involved in these separate pathways were identified (Fig. 9.2) [23, 24]. Tissue factor was identified as the “active” factor in the added tissue extract, and was demonstrated to activate factor VII in the first part of the extrinsic pathway. The intrinsic pathway, sometimes also called the contact activation pathway, was found to involve serial activation of the coagulation factors XII, XI and IX, with factor VIII acting as a co-factor for the latter. Both extrinsic and intrinsic pathways were found to then converge on the “common pathway” involving factor X, prothrombin (factor II), finally leading to the conversion of fibrinogen to fibrin by thrombin. The concept of the two separate pathways was reinforced by the fact that the most widely utilised laboratory assays of coagulation evaluated the extrinsic (the prothrombin time or PT assay) and intrinsic pathway (the activated partial thromboplastin time or aPTT) separately, with both assays impacted by common pathway defects.

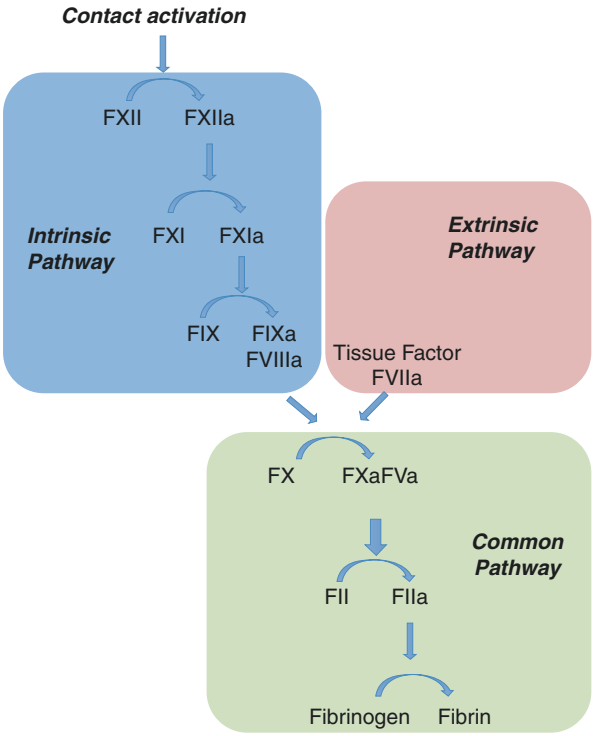


**Fig. 9.2** The extrinsic and intrinsic pathways of coagulation

This concept that two separate independent pathways of sequential enzyme activation could lead to thrombus formation was for a long period of time a central tenet of understanding of the coagulation system and was known as the "waterfall" or "cascade" hypothesis of coagulation [23, 24]. It however became clear with time that the above model was unlikely to reflect physiological coagulation. The observation that inherited factor XII deficiency was not associated with a bleeding tendency raised questions regarding the physiological role of the intrinsic pathway [25]. It was also demonstrated that activated factor VII, or factor VIIa, had the ability to activate factor IX as well as factor X, and therefore that cross-communication between the pathways was likely [26]. With increasing knowledge of the role of the cell surface proteins in the coagulation process, and in particular the role of platelets, a cell-based model of haemostasis then emerged [27] This model divides the coagulation cascade into the separate steps of initiation, amplification, and then propagation (Fig. 9.3).

### *9.5.1 Initiation*

Tissue factor (TF) is a transmembrane protein that is constitutively expressed on the surface of most non-vascular cells, including those located in the subendothelium. Exposure of cells expressing tissue factor to circulating blood is accepted as being

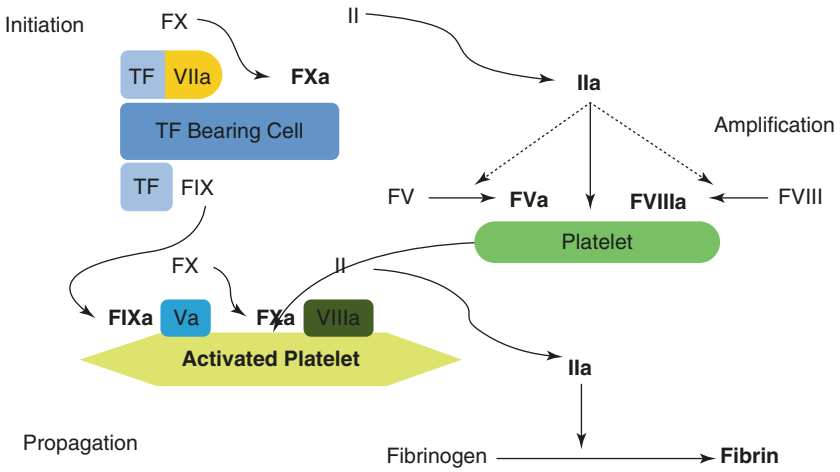


**Fig. 9.3** Cell based model of haemostasis

the physiological trigger of coagulation. There is also evidence that tissue factor expression can be induced in the setting of inflammation on the surface of monocytes, and that microparticles derived from monocytes may also express TF in pathological states [28].

Upon exposure to circulating blood, TF can bind to both factor VII or factor VIIa, with approximately 1% of FVII circulating in the active form [29] FVII not already activated, is rapidly activated to FVIIa and the resulting TF/FVIIa enzymatic structure is known as the extrinsic tenase complex. Within the complex TF acts as a cofactor for VIIa, greatly potentiating the latter's capacity to convert factor X to factor Xa, and, to a lesser degree, factor IX to factor IXa.

The activated factor Xa formed by the extrinsic tenase complex then binds to the surface of the tissue factor-expressing cell and converts a small amount of prothrombin (factor II) to thrombin. This thrombin diffuses away, moving to the surface of nearby platelets leading to both platelet activation and the formation of FXIa, FVIIIa and FVa. These activated proteases then bind to the surface of activated platelets and are central to the amplification phase of coagulation as described below [30].

### *9.5.2 Amplification*

The small amount of thrombin formed during the initiation stage of coagulation is insufficient to convert adequate amounts of fibrinogen to fibrin to form a stable thrombus that is resistant enough to fibrinolytic activity to allow healing to occur. It is however sufficient enough to be responsible for the subsequent amplification of the coagulation cascade. The thrombin produced results in;

1. Further local activation of platelets resulting in a suitable phospholipid surface on which the reactions of the coagulation cascade can proceed,
2. Activation of the co-factors factor V and factor VIII that then localize on the nearby surface of activated platelets,
3. Activation of factor XI that also binds locally to the platelet surface [31].

### *9.5.3 Propagation*

Following the activation of the co-factors and their localization on the platelet surface, the stage is set for the formation of highly efficient enzymatic complexes that are responsible for the burst of thrombin generation that leads to clot formation. Factor IXa formed during the initiation step, binds to factor VIIIa on the platelet surface to form the intrinsic tenase complex. This then efficiently converts factor X to factor Xa, with the latter then binding to its co-factor, factor Va, to form the prothrombinase complex responsible for the effective conversion of prothrombin to thrombin. Factor XIa produced during amplification activates further factor IX, further reinforcing the haemostatic process [27].

The burst of thrombin generated during propagation then cleaves the fibrinopeptides a and b from soluble fibrinogen to form insoluble fibrin monomers. The transglutaminase Factor XIII, itself activated by thrombin, then forms bonds between separate fibrin monomers to form a firm network of cross-linked fibrin that is a requirement for stable thrombus formation [32].

### *9.5.4 Other Roles of the Contact Activation System*

It has been increasingly recognized that there are complex interactions between the contact pathway of the coagulation system with complement and the inflammatory response [33]. FXII-mediated activation of prekallikrein to kallikrein leads to bradykinin production, after kallikrein cleaves high-molecular weight kininogen (HMWK). Kallikrein also cleaves several complement proteins including C3, C5 and factor B, leading to complement activation. Further evidence of the interaction between the two pathways is demonstrated by that fact the multi-ligand binding protein gC1qR can both activate the classical complement pathway by binding C1q, and the contact activation pathway by activating HMWK and FXII. There is emerging interest in manipulating the molecules of the contact activation pathway as a means of controlling the inflammatory response without increasing bleeding risk.

### *9.5.5 Natural Inhibitors of Coagulation*

Normal coagulation is kept in check by several regulatory processes that cause thrombin production to plateau and then diminish, preventing localized activation of coagulation from becoming an inappropriately widespread activation of the clotting cascade.

The initiation phase of coagulation is regulated by tissue factor pathway inhibitor (TFPI), a protein produced by endothelial cells [34]. After a sufficient local concentration of FXa is generated in the initiation step of coagulation, TFPI is able to form an inhibitory quaternary complex with FXa, FVIIa, and tissue factor, effectively turning off the initiation phase of coagulation. Interestingly FV has been demonstrated to bind to TFPI and protect against premature clearance of the inhibitor, suggesting an anticoagulant potentiating role for the unactivated form of FV [35].

Central to regulation of the propagation phase of the coagulation cascade is the protein C anticoagulant pathway that involves protein C and protein S, both vitamin K dependent plasma glycoproteins synthesized in the liver [36, 37]. Thrombin itself initiates this inhibitory pathway after binding to thrombomodulin, a transmembrane protein located on the intact endothelial cell surface in all vascular beds particularly in the microcirculation. Binding of thrombin to thrombomodulin results in a change in substrate specificity that favours thrombin mediated cleavage of the vitamin K dependent protein C to its activated form activated protein C (APC) [38]. Binding of thrombin to thrombomodulin therefore results in its net enzymatic effect being switched from pro-coagulant to anti-coagulant. Another endothelial transmembrane protein, the endothelial protein C receptor (EPCR) binds protein C, helping to localize the protein at the endothelial surface potentiating activation by thrombomodulin bound thrombin. Once activated, APC diffuses away from EPCR and binds to the extrinsic tenase and prothrombinase complexes where it acts to inactivate factor VIIIa and factor Va respectively. Protein S acts as a co-factor for protein C in these reactions, as well as having some direct anticoagulant activity [39]. Protein S has recently been shown to help with localization of TFPI on phospholipid surfaces, increasing the efficiency of inhibition of FXa by TFPI. It appears clear that protein S exerts its anticoagulant effect by more than one mechanism. In plasma, PS circulates both free (40%) and bound to the C4b-binding protein (60%). It is the free form of PS that has cofactor activity [37].

Finally antithrombin (AT) is a single chain plasma glycoprotein that belongs to the serine protease inhibitor superfamily (serpins). It plays a central role in the inactivation of circulating activated clotting factors, forming a 1:1 complex that is cleared by the liver. It is the main physiological inhibitor of thrombin and also binds to factors Xa, IXa, XIa, and XIIa [40]. Thrombin inhibition by AT is potentiated more than 1000-fold by heparin, due to conformational change of the AT molecule upon heparin binding, and it is this mechanism that results in heparin's activity as an anticoagulant agent [41]. AT activity is also enhanced by heparan sulfates that are present on intact endothelial surfaces, one of many mechanisms that help to restrict the activation of coagulation to the site of vascular injury [35].

Inherited deficiency states of the main inhibitory proteins of coagulation, namely protein C, protein S and antithrombin, have all been described, and result in a significant pro-thrombotic tendency. Such deficiency states are relatively rare accounting, when combined, for less than 5% of individuals with venous thrombosis in a Caucasian population. A mutation in FV known as the FV Leiden mutation (Arg506Gln), which prevents proteolysis at one of the APC cleavage sites is far more common occurring in ~5% of Caucasians but results in a much milder prothrombotic condition.

## 9.6 Fibrinolysis

The fibrinolytic system is responsible for the dissolution of thrombus composed of cross-linked fibrin, and plays a major role in helping maintain a patent vascular system [42]. It is composed of a number of enzymes, most of which are serine proteases, that act in concert to convert insoluble fibrin to soluble fibrin degradation products (FDPs). The central protein of the fibrinolytic system is plasminogen, a single-chain glycoprotein consisting of 791 amino acids, which is converted to its active form plasmin by the cleavage of a single Arg561-Val562 peptide bond [43]. Tissue-type plasminogen activator (tPA) is the physiological activator of plasminogen on the thrombus surface. Activation of plasminogen to plasmin is potentiated in the presence of fibrin due to the fact that both plasminogen and tPA bind to lysine residues on the surface of fibrin, being brought into close proximity to each other, allowing plasminogen activation. Both tPA and another plasminogen activator, urokinase-type plasminogen activator, play a role in the activation of plasminogen that is bound to the endothelial cell surface.

Once activated, plasmin cleaves fibrin into soluble fibrin degradation products, of which D-dimer is one. D-dimer consists of two cross-linked fibrin D-domains and is not normally present in the absence of recent plasmin activity. It is therefore used as a laboratory marker of active thrombosis and is a sensitive test that can be used to rule out recent venous thromboembolism.

Like the coagulation cascade, the fibrinolytic system also has a number of inhibitory proteins that in normal circumstances prevent widespread activation of fibrinolysis. Plasminogen activator inhibitor-1 (PAI-1) is a 52-kd, single-chain glycoprotein that belongs to the serpin family, and is the main inhibitor of both tPA and uPA, doing so by forming a 1:1 complex that is cleared by the liver [44]. Circulating plasmin is quickly mopped up by $\alpha_2$-plasmin that is present in the circulation at a high concentration. The most recently described inhibitor of fibrinolysis is thrombin-activatable fibrinolysis inhibitor (TAFI), a carboxypeptidase [45]. TAFI is activated by thrombin, a process that is markedly accelerated if thrombin is bound to thrombomodulin. The antifibrinolytic activity of TAFI is due the fact that it cleaves C-terminal lysine and arginine residues from fibrin. This significantly reduces the binding of plasminogen to fibrin, therefore decreasing the activation of plasminogen by tPA on the surface of the fibrin clot.

The fibrinolytic system is manipulated therapeutically by administration of either naturally occurring (streptokinase) or recombinant protein (r-tPA) plasminogen activator that exert the same effect as endogenous tPA, leading to activation of plasmin and resulting thrombus lysis.

## 9.7 Conclusions

Primary and secondary haemostasis both involve carefully balanced systems that if disturbed can lead to issues with either bleeding or pathological thrombosis. An improved understanding of the molecular processes involved has led to the

development of more targeted therapeutic options, such as the direct thrombin inhibitors and direct factor Xa inhibitors, with the aim of increasing the benefit and reducing the risks associated with anticoagulation. Increasing recognition of the interaction between the inflammatory and coagulation pathways may lead to novel therapeutic targets to control the inflammatory response. Continued advances in our understanding of the relationship between the structure and function of the proteins and receptors involved in haemostasis, along with improved technology, is likely to lead to further therapeutic advances in coming decades.

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