*Part 1*

# The laboratory interface

# Chapter 1
# Basic principles underlying the coagulation system

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## Introduction

The coagulation system, incorporating pro- and anticoagulant proteins, the fibrinolytic system, platelets and the vascular endothelium, is a critical system in homeostasis. An efficient system to repair defects in the vessel wall is clearly beneficial but thrombosis in critical sites can also be detrimental to normal physiology, hence the highly developed anticoagulant and fibrinolytic systems. The importance of elements of the system is indicated by the presence of homologous proteins in primitive organisms and studies suggest that these proteins evolved at least 450 million years ago.

Coagulation factors also have a role in normal embryonic growth and development, and complete deficiencies in tissue factor (TF), factor VII (FVII), tissue factor pathway inhibitor (TFPI), factor X (FX), factor V (FV), prothrombin (PT) or protein C (PC) result in embryonic lethality in transgenic mice.

In recent years, the interplay between coagulation and inflammation/sepsis has been increasingly recognized with a number of anticoagulant proteins associated with pronounced anti-inflammatory effects. PC and antithrombin (AT) have both been used effectively in the treatment of sepsis and improvements in mortality rates are seen with infusions of activated PC which are independent of pretreatment levels.

## The current model of coagulation

Formation of a fibrin clot is the final result of a complex series of proteolytic events.

Previous models of coagulation suggested that the intrinsic and extrinsic coagulation cascades functioned independently. It is now understood that there are two phases of coagulation, which are intimately linked:

**1** initiation, and
**2** propagation.

Thrombin plays a central part:

**1** in the activation of factors and cofactors that promote further thrombin generation;
**2** in the activation of anticoagulant factors that extinguish thrombin generation; and
**3** in determining the balance of fibrinolysis.

The role of platelets in both primary hemostasis (generation of the platelet plug) and secondary hemostasis (formation of a fibrin clot) is also recognized.

## Platelets

Platelets are central to primary hemostasis. They are responsible for the initial closure of the defect in the vessel wall through the formation of the platelet plug. Important pro- and anticoagulant factors are stored within platelet granules and these are released into the microenvironment around vessel injury. In secondary hemostasis, platelets provide the membrane surface to which the activated clotting factors bind, leading to increased enzyme efficiency and increased thrombin generation.

Platelets are produced from megakaryocytes in the bone marrow and are released into the circulation. The platelet count is in the range $150–400 \times 10^3$/L in normal individuals and the circulating half-life is approximately 10 days.

The platelet structure has a number of specialized features:

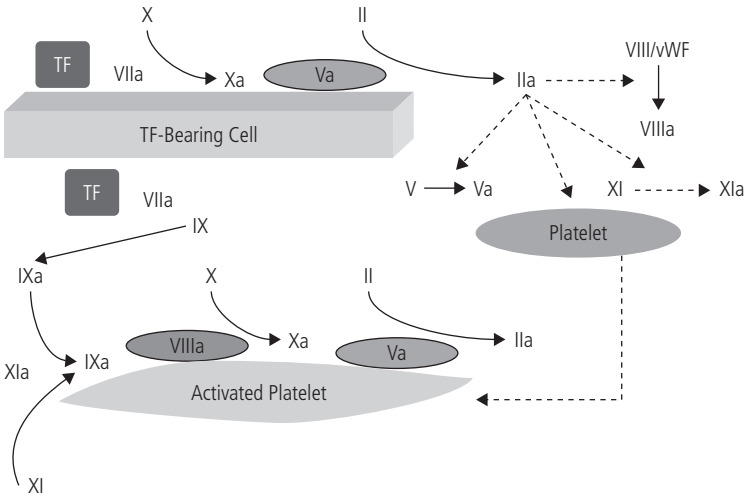


**Figure 1.1** Initiation and propagation of coagulation.

• Surface glycoproteins (Gp) are present on the platelet membrane, which enable binding to collagen and von Willebrand factor (VWF) on the subendothelial surface (Gp Ia-IIa, Gp Ib/IX/V), to fibrinogen (Gp IIb-IIIa) and to other platelets via thrombospondin (Gp IV).
• The membrane surface is invaginated, increasing the surface area for release of the contents of intracellular granules.
• Within the platelet, alpha granules contain a variety of hemostatic proteins including FV, VWF, fibrinogen and fibrinolytic proteins. Dense granules contain adenosine diphosphate and triphosphate as well as serotonin.
• The dense tubular system mediates calcium flux.
• Peripheral microtubules and cytoplasmic filaments are present, which control platelet shape change.

On vessel injury and exposure of the subendothelium, circulating platelets bind via the surface glycoproteins, Gp Ia-IIa and Gp Ib/IX/V. The platelets then undergo shape change, becoming spherical and extending pseudopods.

The intracellular granules are moved towards the surface and release of their contents into the microenvironment is mediated by the mobilization of calcium. Two metabolic pathways govern both the mobilization of calcium and the activation of platelets.

Activation of platelets results in exposure of Gp IIb-IIIa and binding to fibrinogen.

Platelet aggregation leads to a "flip" in the platelet membrane with exposure of the procoagulant phosphatidylserine which provides the surface for binding of the coagulation factors and secondary hemostasis.

## Coagulation

### Step 1: Initiation

In the current model of coagulation, initiation of clotting occurs when TF binds to activated factor VII (FVIIa) (Figure 1.1).

TF is constitutively expressed on cells such as smooth muscle cells and fibroblasts but not on resting endothelium. TF is exposed to the circulating blood by disruption of the endothelium or by activation of endothelial cells or monocytes.

Approximately 1–2% of circulating FVII exists in the active form, FVIIa, but this serine protease is not catalytically active until it binds to TF.

The FVIIa–TF complex activates both FIX and FX although FX is a more efficient substrate for the complex.

Activated FX (FXa) binds to the platelet membrane and cleaves prothrombin to thrombin. The small amount of thrombin initially formed activates a number of factors that are critical for the second phase of coagulation, propagation; namely factors V, VIII and XI.

Another crucial role is the activation of platelets

**Table 1.1** Procoagulant clotting factors.

| *Current nomenclature* | *Name* | *Function* | *Half-life (h)* |
|---|---|---|---|
| Factor I | Fibrinogen | Precursor of fibrin | 90 |
| Factor II | Prothrombin | Serine protease in prothrombinase complex | 65 |
| (Factor III) | Calcium | Cofactor | |
| (Factor IV) | Tissue factor | Initiation of coagulation | |
| Factor V | Proaccelerin | Cofactor in prothrombinase complex | 15 |
| Factor VII | Proconvertin | Initiation of coagulation | 5 |
| Factor VIII | Antihemophilic factor | Cofactor in tenase complex | 12 |
| Factor IX | Christmas factor | Serine protease in tenase complex | 24 |
| Factor X | Stuart–Prower factor | Serine protease in prothrombinase complex | 40 |
| Factor XI | Plasma thromboplastin antecedent | Amplification of coagulation | 45 |
| Factor XII | Hageman factor | Contact factor | 50 |
| Factor XIII | Fibrin stabilizing factor | Cross-linkage of fibrin | 200 |
| Prekallikrein | Fletcher factor | Contact factor | 35 |
| High molecular weight kininogen | Fitzgerald factor | Contact factor | 150 |

that provide the surface on which the propagation phase of coagulation occurs.

#### Step 2: Propagation of coagulation

Propagation of coagulation depends on the presence of sufficient concentrations of activated serine proteases, cofactors and platelets from the initiation phase (Table 1.1).

Whereas initiation of coagulation depended on the TF–FVIIa complex, propagation depends on two complexes:

**1** the intrinsic factor tenase; and
**2** the prothrombinase complexes.

These complexes have unique specificity but many common features. All consist of a vitamin K-dependent serine protease with an accessory cofactor bound to a membrane. The proteins are structurally similar and the assembly of the complexes increases enzymatic efficiency over the efficiency of the serine protease alone.

##### *The intrinsic tenase complex*

In the intrinsic tenase complex, membrane-bound FIXa forms a complex with its cofactor FVIIIa and calcium. This complex is the major activator of FX and is 50 times more active than the FVIIa–TF complex. In comparison to the FIX serine protease alone, the catalytic efficiency of the intrinsic tenase complex is increased by 5–6 orders of magnitude. More than 90% of the FXa produced in the coagulation cascade is produced by the intrinsic tenase complex. Additional FIX is generated by activated FXI to further augment FXa and thrombin generation.

##### *The prothrombinase complex*

The prothrombinase complex is formed when FXa binds to the membrane surface by its Gla domain and complexes with its cofactor FVa and calcium. The prothrombinase complex is 300,000-fold more active than FXa alone in catalyzing the conversion of prothrombin to thrombin. The rate-limiting component of the propagation phase is the concentration of FXa. The consequence of the interplay of the activated factors and cofactors in the propagation phase of coagulation is that 96% of the total thrombin generated is produced in this phase.

### Natural anticoagulants

The natural anticoagulants (Table 1.2) play a critical part in controlling thrombin generation:

**Table 1.2** Natural anticoagulants.

| *Protein* | *Function* |
|---|---|
| Antithrombin | Inhibits thrombin, FXa, IXa, XIa |
| Protein C | Inhibits FVIIIa and FVa |
| Protein S | Cofactor for protein C |
| TFPI | Inhibits TF/FVIIa |
| Thrombomodulin | Activates protein C and TAFI in a complex with thrombin |
| Heparin cofactor 2 | Inhibits thrombin |
| $\alpha_2$-Macroglobulin | Inhibits thrombin and FXa |
| $\alpha_1$-Antitrypsin | Inhibits FXIa and other serine proteases |
| Protease nexin 2 | Inhibits FXIa |

TFPI, tissue factor pathway inhibitor.

- TFPI is an efficient inhibitor of the FVIIa–TF complex by formation of a quaternary complex with FVIIa/TF/FX, leading to rapid extinction of the initiation phase.
- AT effectively neutralizes all procoagulant serine proteases and is present at twice the concentration of its substrates. Antithrombin is a potent inhibitor of thrombin, FIXa, FXa and FXIa.
- Both AT and TFPI are stoichiometric inhibitors.

There is also a dynamic inhibitory system in the thrombomodulin–PC system. The larger amount of thrombin generated in the propagation phase (96%) binds to thrombomodulin and this complex activates PC to activated PC (aPC).

- aPC with its cofactor protein S (PS) inhibits FVa and FVIIIa, thus switching off thrombin generation by the intrinsic tenase and prothrombinase complexes.
- $\alpha_2$-Macroglobulin inhibits serine proteases by steric hindrance rather than by inactivation of the active site. It is responsible for approximately 20% of the inhibition of thrombin and 10% of the inhibition of FXa. It is an acute phase reactant and may be a significant inhibitor of coagulation when levels are elevated. This is particularly important in children where the level of $\alpha_2$-macroglobulin is higher.
- Protease nexin 2 and $\alpha_1$-antitrypsin, inhibitors of FXIa and the serine proteases, respectively.
- Heparin cofactor II, a specific inhibitor of thrombin.

## The fibrinolytic system

The role of the fibrinolytic system is to ensure that the formation of the fibrin clot is localized to the site of vessel injury and that the clot is efficiently removed once wound healing has occurred (Figure 1.2). As the fibrin clot is formed, pro- and anti-fibrinolytic proteins are incorporated into the clot by binding to fibrin (Table 1.3). Plasminogen, plasmin and tissue plasminogen activator (tPA) bind to lysine residues on fibrin while $\alpha_2$-antiplasmin is cross-linked to fibrin by FXIII.

Two forms of plasmin(ogen) occur:

**1** Glu-plasmin(ogen) has a glutamate residue at position 1 of the protein and is less active than

**2** Lys-plasmin(ogen), which has lysine as the first amino acid after removal of the first 76 amino acids by catalytic degradation.

Plasminogen is cleaved to form plasmin by:

- tPA, which is released by endothelial cells; and
- urokinase (uPA), which is primarily found in urine.

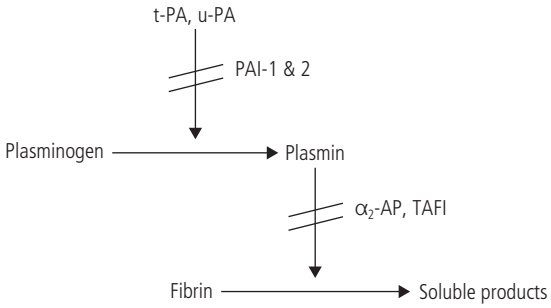


**Figure 1.2** The fibrinolytic system.

**Table 1.3** Fibrinolytic proteins.

| *Antifibrinolytic proteins* | *Profibrinolytic proteins* |
|---|---|
| $\alpha_2$-Antiplasmin | Plasminogen |
| PAI-1 | Plasmin |
| PAI-2 | tPA |
| TAFI | uPA |

PAI, plasminogen activator inhibitor; TAFI, thrombin activatable fibrinolysis inhibitor; tPA, tissue plasminogen activator; uPA, urokinase.

The half-life of tPA in plasma is short because of rapid inactivation by its specific inhibitor plasminogen activator inhibitor type 1 (PAI-1) and clearance by the liver. However, the activity of fibrin bound tPA for fibrin-bound plasminogen is markedly enhanced and is protected from inhibition.

Both fibrin and fibrinogen are substrates for plasmin, which hydrolyzes arginine and lysine bonds at multiple sites resulting in cleavage products known as fibrin(ogen) degradation products (FDPs). However, only FDPs that are derived from cross-linked fibrin are detectable as D-dimers. D-dimers therefore are specific for clot-bound fibrin.

### Major inhibitors of fibrinolysis

These are PAI-1 and plasminogen activator inhibitor type 2 (PAI-2), $\alpha_2$-antiplasmin, $\alpha_2$-macroglobulin and thrombin activatable fibrinolysis inhibitor (TAFI):

- PAI-1 and PAI-2 are found in platelets and endothelial cells.
- PAI-2 is produced in increasing amounts by the placenta during pregnancy but is also present in monocytes.
- Synthesis of $\alpha_2$-antiplasmin and TAFI occurs in the liver.

Circulating plasmin is rapidly inhibited by $\alpha_2$-antiplasmin, augmented by $\alpha_2$-macroglobulin. Plasmin bound to fibrin is relatively protected from inhibition but the process of plasmin binding to fibrin can be inhibited by $\alpha_2$-antiplasmin, especially if it is cross-linked to fibrin by FXIII. TAFI is activated by the thrombin–thrombomodulin complex and it removes lysine residues from fibrin, thus protecting it from degradation by plasmin.

## Further reading


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