

22.7 Haemostasis 5490

22.7.1 The biology of haemos

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22.7.1 The biology of haemostasis and thrombosis 5490 Gilbert C. White, II, Harold R. Roberts, and Nigel S. Key

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ESSENTIALS Haemostasis—a component of the wound defence mechanism—is a process by which vessel wall components and platelets act in concert with procoagulant and anticoagulant proteins to form a plug of cells and cross-linked fibrin. The plug is later remodelled and replaced by new tissue as part of wound healing. These processes are very complex and

involve highly controlled pathways of interaction between cells, glycans, and membrane-bound and soluble proteins of coagulation and fibrinolysis, as well as their cognate inhibitors.

Thrombosis—this is an abnormal state leading to formation of a clot obstructing blood vessel flow; dislodgement leads to thromboembolism. **Blood vessel wall** **Vascular endothelial cells**—these make many contributions to haemostasis by (1) regulating vascular tone—through production of (a) vasodilators, most notably nitric oxide and prostacyclin (PGI₂), and (b) vasoconstrictors, particularly endothelin and angiotensin 2; (2) exerting anticoagulant effects—through production of PGI₂, nitric oxide, thrombomodulin, tissue factor (TF) pathway inhibitor, glycosaminoglycans, CD39, and tissue plasminogen activator (tPA); (3) promoting procoagulant effects—the dominant effect of endothelial cells is anticoagulant, but they store/produce von Willebrand factor and TF; and (4) up-regulating expression of receptors—including thrombin receptors, thrombomodulin, and endothelial cell protein C receptor, and a number of adhesive receptors that are important for the interaction of leucocytes and the vessel wall. **Other elements**—these include (1) extracellular matrix—promotes platelet adhesion, cellular migration, cell proliferation, and endothelial and smooth muscle cell interactions; (2) smooth muscle cells; and (3) adventitia. **Platelets** Platelets are key components of the haemostatic plug. They adhere to damaged vessels where subendothelial matrix is exposed, aggregating to form an initial plug that prevents blood loss by occluding the breach in the vessel wall. Their involvement in haemostasis can broadly be divided into the following processes: (1) platelet adhesion—accomplished by a number of glycoproteins and other adhesion receptors on the platelet surface; (2) platelet activation—following adhesion and in response to soluble agonists, platelets undergo reactions (including changes in metabolism of membrane inositol phospholipids) that lead to generation of platelet coagulant activity, thrombin, and release of ADP, which lead to activation of additional platelets; and (3) platelet aggregation—mediated by binding of activated platelet surface glycoprotein α IIb- β 3 to fibrinogen or fibrin, which by virtue of its dimeric structure can bind to more than one platelet and thereby facilitate their aggregation, which serves to localize the haemostatic plug at the site of injury. **Blood coagulation** Blood coagulation depends on the presence of serial proenzymes that are sequentially activated in the presence of activators and cofactors, with key elements being (1) TF—this is constitutively produced in several extravascular cell types such as fibroblasts and smooth muscle cells, but not in cells exposed to the circulating blood; it functions as a receptor for factor VII and initiates the blood coagulation pathway after it binds to and activates factor VII; (2) TF-VIIa complex—this activates factors IX and X which, in the presence of their respective cofactors (VIII and V), rapidly convert prothrombin

22.7.1 The biology of haemostasis and thrombosis 5491 (factor II) to thrombin; (3) thrombin converts soluble fibrinogen to fibrin; and (4) fibrin undergoes cross-linking by activated factor XIII to form the stable haemostatic plug. Important aspects of the system include (1) platelets which provide the surface for activated clotting factors, leading to the explosive generation of thrombin and subsequent clot formation; and (2) the initial generation of relatively small amounts of thrombin is essential for feedback activation of factors V, VIII, XI, and XIII, as well as of platelets. **Inhibitors of the coagulation reactions**—there are numerous inhibitors of the reactions involved in blood coagulation, which are essential for the temporal control and safety of the process. These include (1) TF pathway inhibitor—occurs in forms free within the circulation and anchored to platelets and endothelial cells; inhibits the VIIa-TF-Xa complex; (2) antithrombin—a serpin inhibitor of thrombin, factor X, and other proteases; (3) other inhibitors—these include α 1-antitrypsin, C-1 esterase inhibitor, and protein Z-dependent protease inhibitor. The

fibrinolytic system The fibrinolytic system depends on the activation of plasminogen adsorbed on the fibrin surface by tPA to form plasmin, which de-grades fibrin to form specific fibrin degradation products, and when generated in excess also degrades fibrinogen, factors VIII and V, and von Willebrand factor. Important aspects of the system include (1) free plasmin in the circulation is rapidly inhibited by α_2 -antiplasmin; (2) plasminogen and tPA associate in the circulation with fibrinogen, hence when fibrinogen is converted to fibrin, the clot is rich in both of these proteins, which are protected from the inhibitory action of antiplasmin, hence clots can be lysed without interference from inhibitors; (3) many other regulatory mechanisms exist, including plasminogen activator inhibitor 1, urokinase plasminogen activator, and thrombin-activatable fibrinolytic inhibitor. The balance of fibrinolysis and coagulation Fibrinolysis and coagulation are interrelated: fibrin clots are normally lysed by plasmin locally released from plasminogen by the action of tPA, and this process can be enhanced by some procoagulant factors (e.g. activated factor XII, and protein C). This system, so delicately controlled and normally maintained in a dynamic equilibrium, is strongly influenced by components involved in inflammatory and other defence mechanisms in the host. An integrated understanding of these processes offers the potential for improved means to predict the adverse complications of many diseases and ultimately to prevent their occurrence.

Introduction Fluid blood is contained within the vascular tree, but as a result of minor trauma that occurs during the wear and tear of everyday living, leaks occur in the blood vessel wall that must be sealed by a solid impermeable fibrin clot in order to prevent significant blood loss. The clot is formed from clotting factors in flowing blood and is located and restricted to the site of the leak without dissemination throughout the vascular tree. This is the process of haemostasis, an exquisitely controlled mechanism that requires components of the vessel wall, blood platelets, and soluble procoagulant and anticoagulant proteins. The haemostatic plug consists of a mass of platelets, red blood cells, and leucocytes enmeshed in interlocking strands of insoluble and cross-linked fibrin fibres that plug the leak. Once formed, the haemostatic plug is gradually replaced by new tissue as a part of wound healing. This process requires lysis of the blood clot by the fibrinolytic system and subsequent ingrowth of new cells. Thus, haemostasis is not an isolated phenomenon, but is one component of the defence mechanisms that lead to eventual wound healing. Thrombosis, as opposed to haemostasis, is a pathological state in which a clot is formed that partially or completely obstructs the flow of blood within the blood vessel and sometimes dislodges to become an embolus. To understand the biology of haemostasis and thrombosis, it is necessary to know the roles of the vessel wall, the platelets, the coagulation and fibrinolytic systems, and their respective inhibitors.

Blood vessel wall The anatomy of the wall of both an artery and a vein is shown schematically in Fig. 22.7.1.1. All blood vessels are lined by an intima consisting of a monolayer of endothelial cells that rest upon a loose network of tissue called the extracellular matrix. In addition to the intima, larger and intermediate sized arteries contain two other layers: the media, composed mostly of smooth muscle cells, and the adventitia, consisting largely of connective tissue, nerves, and nutrient vessels. These three layers also exist in veins, but the media and adventitia are much less distinct and are not visible in the smaller arterioles, venules, and capillaries.

Endothelium Internal elastic lamina Intima External elastic lamina Media Adventitia Fig. 22.7.1.1 Schematic diagram of a vessel wall consisting of the intima, the media (smooth muscle cells), and the adventitia. The intima consists of a layer of endothelium that is exposed to the circulating blood. The subendothelial matrix lies below the endothelium and is separated from the media by the internal elastic membrane. See text for detailed description of each layer.

section 22 Haematological disorders 5492 Endothelial cells Endothelial cells form the basis of vascular development and are derived from embryonic mesoderm. Embryonic endothelial cells (angioblasts) develop under the influence of growth hormones including basic fibroblast growth factor (b-FGF) and vascular endothelial growth factor (VEGF), both of which interact with receptors on the cell membrane termed receptor tyrosine kinases. These early blood vessels expand into a vascular tree under the influence of two major hormones, angiopoietin 1 and 2, that bind to a family of tyrosine kinase receptors called tie-1 and tie-2 (tyrosine kinase plus Ig and epidermal growth factor-like domains) on endothelial cells. To fully develop into an intact vascular tree, endothelial cells must interact with the extracellular matrix and other cells, a process that requires cell-cell adhesion that is dependent upon cell surface cytoadhesive molecules (CAMs) such as platelet-endothelial cell adhesion molecule-1 (PECAM-1), and vascular endothelial cell cadherin (VE-cadherin). Endothelial cell structure is also dependent upon the integrin family of molecules and interactions with the extracellular matrix. Endothelial cells are heterogeneous in appearance, function, and genetic regulation. In the brain, endothelial cells form very tight junctions with one another to preserve the blood-brain barrier; in the spleen and liver, the interendothelial gaps are wide, permitting soluble and cellular trafficking between blood and the extravascular space. Not all endothelial cells synthesize the same proteins. Tissue plasminogen activator (tPA) is synthesized by only about 3% of cells. Even von Willebrand factor (VWF), often regarded as a specific marker for endothelial cells, is not expressed in all cells. The microenvironment also plays an important role in regulating endothelial cell function.

Haemodynamic forces, including hydrostatic pressure, and shear stresses and strains can influence endothelial cell structure and function. Haemodynamic forces can even regulate endothelial cell gene expression. For example, there is a shear-stress response element in the gene governing the synthesis of the β chain of the platelet-derived growth factor (PDGF). Other endothelial cell genes responsive to shear forces include those coding for tPA, intercellular adhesion molecule (ICAM), and vascular cell adhesion molecule-1 (VCAM-1). Endothelial cells contribute to haemostasis by their contributions to vascular tone and procoagulant, anticoagulant, fibrinolytic, and antifibrinolytic activities. Vascular tone Vasoregulatory substances produced by endothelial cells are shown in Table 22.7.1.1. The most important vasoregulators are nitric oxide, previously known as endothelial cell-derived relaxation factor (EDRF), and prostacyclin. Nitric oxide and prostacyclin are also important antiplatelet agents. On the other hand, the most important vasoconstrictors are endothelin and angiotensin 2. Endothelin is also a mitogen for smooth muscle cells. Anticoagulant properties

The anticoagulant properties of the endothelial cells are shown in Table 22.7.1.2. Prostacyclin not only causes vasodilation, but it is a potent inhibitor of platelet aggregation. Nitric oxide has a similar effect. An important anticoagulant function of endothelial cells is the expression of thrombomodulin, a transmembrane-bound protein that acts as a receptor for thrombin. The thrombomodulin-thrombin complex is the physiological activator of protein C. Activated protein C, in turn, inactivates clotting factors Va and VIIIa to turn off coagulation. The Table 22.7.1.1 Vasoregulatory substances produced by endothelial cells Vasoregulatory substance Action Vasodilators Nitric oxide (NO) $\uparrow$ cGMP in SMC Prostacyclin (PGI₂) $\uparrow$ Cyclic AMP in platelets Monoamine oxidase (MAO) $\downarrow$ Catecholamines Vasoconstrictors Endothelin Activates Ca²⁺ channels in SMC Angiotensin 2 Converts angiotensin 1 to 2 by ACE Prostaglandin G₂,H₂(PGG₂, PGH₂) Acts on SMC ACE, angiotensin-converting enzyme on endothelial cells; AMP, adenosine monophosphate (converted to adenosine); SMC, smooth muscle cells. Table 22.7.1.2 Procoagulant and anticoagulant properties of endothelial cells Procoagulant and anticoagulant Synthesis Action Procoagulants von Willebrand factor (VWF) Constitutive Carrier of Factor VIII; platelet adhesion to

vessel wall Tissue factor (TF) Inducible Receptor for Factor VII Anticoagulants Prostacyclin (PGI₂) Constitutive Inhibits platelet aggregation Nitric oxide (NO) Constitutive Vasodilation Thrombomodulin (TM) Constitutive TM/thrombin complex, activates protein C Tissue factor pathway inhibitor (TFPI) Constitutive Inhibits TF-VIIa-Xa complex Glycosaminoglycans (GAGs) Constitutive Antithrombins CD 39 (ectonucleotidase) Constitutive Degrades ATP and ADP and inhibits platelet aggregation Tissue plasminogen activator (tPA) Constitutive Converts plasminogen to plasmin

22.7.1 The biology of haemostasis and thrombosis 5493 action of the thrombin-thrombomodulin complex is enhanced when protein C occupies the endothelial cell protein C receptor (EPCR), also located on the endothelial surface (see 'Receptors'). Endothelial cells contribute to the control of coagulation by synthesizing tissue factor pathway inhibitor, which inhibits the tissue factor-mediated initiation of the clotting reactions. They also synthesize glycosaminoglycans such as heparan sulphate and other proteoglycans that inhibit thrombin via their interaction with antithrombin. In addition, they express vascular ectonucleoside triphosphate diphosphohydrolase, otherwise known as CD39, on their surface. CD39 acts to convert ATP/ADP to AMP and then to adenosine, which inhibits platelet aggregation. Endothelial cells also secrete fibrinolytic factors including prostacyclin and tissue plasminogen activator, among others. Procoagulant properties Although the overall effect of endothelial cells is anticoagulant, these cells do participate in coagulation by storing proteins such as VWF and by synthesizing tissue factor (TF) under certain conditions. Procoagulant properties of the endothelial cell are also listed in Table 22.7.1.2. VWF is synthesized constitutively by endothelial cells and is essential for platelet adhesion to the vessel wall and as a carrier for blood clotting factor VIII. VWF is stored in Weibel-Palade bodies as depicted in Fig. 22.7.1.2. It is released into the circulation in multimers of heterogeneous molecular mass ranging from 1000 kDa to about 20 000 kDa. Endothelial cells also secrete very large VWF multimers abluminally into the extracellular matrix. TF acts as a binding protein for factor VII and is essential for the initiation of coagulation. It is not constitutively produced by endothelial cells, but it can be induced by tissue necrosis factor (TNF), endotoxin, and other inflammatory substances. Receptors The receptor function of endothelial cells plays an important role in haemostasis and thrombosis (Table 22.7.1.3). They express thrombin receptors such as protease-activated receptor (PAR)-1, -2, and -4. Thrombin cleaves the C-terminal end of the receptor, which then binds to the remaining cell-associated protein (a so-called tethered ligand) and triggers intracellular signalling through G proteins, resulting in activation of endothelial cells. PARs influence vascular tone but do so through different intracellular signalling mechanisms. The thrombin-thrombomodulin complex not only activates protein C, but also activates a protein known as the thrombin-activatable fibrinolytic inhibitor (TAFI), a procarboxypeptidase that functions to inhibit fibrinolysis. Endothelial cells also express EPCR that acts to modulate the activity of activated protein C. EPCR resides on the endothelial cell and enhances protein C activation by about 20-fold in vivo. EPCR binds protein C or activated protein C and presents it to the thrombin-thrombomodulin complex. Binding of APC to EPCR is also important in the inflammatory process in that through cell signalling mechanisms it decreases inflammatory cytokines and other molecules involved in inflammation. In addition, EPCR acts as a receptor for coagulation factor VII which binds to EPCR with an affinity similar to that of protein C. Urokinase plasminogen activator receptors are not found on resting endothelial cells, but are found on those involved in angiogenesis. There are a number of adhesive receptors on the surface of endothelial cells, as shown in Table 22.7.1.3. The adhesion of neutrophils is dependent upon the expression of P-selectin. P-selectin is rapidly internalized by the endothelial cell, but this is followed

by expression of another cytoadhesive molecule, E-selectin, which is necessary for continued adherence and rolling of neutrophils along the endothelial cell surface. ICAM and VCAM are receptors for leucocytes and are important for the interaction of leucocytes and the vessel wall.

Extracellular matrix The extracellular matrix is a complex, heterogeneous structure beneath the endothelium with a number of constituents that contribute to haemostasis and thrombosis. The matrix consists of a network of collagens, elastins, proteoglycans, and glycoproteins, including fibronectin, vitronectin, laminin, tenascin, thrombospondin, VWF, and osteopontin, as shown in Table 22.7.1.4. The matrix proteins promote platelet adhesion, cellular migration, cell proliferation, and endothelial and smooth muscle cell interactions. Collagens are the most abundant proteins in subendothelial connective tissue. Collagen types I, II, III, IV, V, VI, and VII have been identified in various matrix tissues. The collagens are synthesized by endothelial cells, smooth muscle cells, and adventitial fibroblasts. The various collagens contribute to the integrity of the vessel wall,

Fig. 22.7.1.2 Electron micrograph of an endothelial cell. Weibel-Palade bodies containing immunogold-labelled multimers of VWF are depicted by the arrows.

Table 22.7.1.3 Receptor function of endothelial cells

Receptor	Ligand	Protease activated receptor 1 (PAR-1)	Thrombin	Thrombomodulin	Thrombin	Protein C	Urokinase plasminogen activator receptor (u-PAR)	Urokinase	Adhesive receptors:	Intercellular cytoadhesive molecule (ICAM)-1, -2	Integrins $\alpha 1\beta 2$; $\alpha m\beta 2$	Vascular cytoadhesive molecule (VCAM) $\alpha 1\beta 2$; $\alpha 4\beta 1$	P-selectin	PSGL-1	E-selectin	ESL-1; PSGL-1; LAMP, Mac2-BP

section 22 Haematological disorders 5494 but they also play a role in platelet activation and, in some instances, coagulation. For example, collagen IV has been shown to be a specific high-affinity binding protein for blood coagulation factor IX, although the function of this complex is not yet known. The proteoglycans constitute a heterogeneous group of molecules composed of a core protein attached to a glycosaminoglycan. These include decorin, biglycan, heparan sulphate, dermatan sulphate, and others. Heparan sulphate, for example, can combine with antithrombin (AT) to inhibit thrombin. The precise role of all of the proteoglycans is not known, but some attach to collagen and are necessary for maintaining the structure of the vessel wall. The matrix also contains elastin, which is secreted by endothelial and smooth muscle cells as tropoelastin that is converted to mature elastin in the matrix where it is assembled into fibres. One function of elastin is simply to maintain the elastic structure of the vessel wall. This substance is found interspersed between smooth muscle cells as well as the matrix. It may also function in cell migration from the vessel wall to the extravascular space. Fibronectin, vitronectin, and laminins are also components of the extracellular matrix which function in fibrinolysis and platelet adhesion. Within the extracellular matrix there are a number of matrix metalloproteinases (MMPs), a group of enzymes useful in matrix degradation and repair. They are secreted as proenzymes and converted to active enzymes that require zinc or calcium as cofactors. They have several functions as listed in Table 22.7.1.5. Their activities in matrix degradation and repair are controlled by tissue inhibitors of metalloproteinases.

Smooth muscle cells The smooth muscle cell layer, found in medium- and larger-sized vessels and more prominently in arteries, has several functions related to the biology of haemostasis and thrombosis. Smooth muscle cells possess contractile, biosynthetic, and proliferative functions. Contractile properties governed by such substances as nitric oxide, prostacyclin, and endothelin play important roles in vasodilation and vasoconstriction, respectively. Smooth muscle cells, like endothelial cells, synthesize growth factors such as VEGF, insulin-like growth factors (IGF), epidermal growth factors (EGF), activins, and others that are important in smooth muscle cell generation. Smooth muscle cell proliferation is a hallmark of the atherosclerotic

lesion. Biosynthetic products of the smooth muscle cells include various types of collagens, elastin, glycoproteins, and proteoglycans. When exposed to injury, smooth muscle cells can also express functionally active TF, contributing to the initiation of blood coagulation.

Adventitia The adventitia is composed of a loose network of cells consisting of fibroblasts, adipocytes, and mast cells. Collagens I and III, glyco- proteins, and elastin are synthesized by fibroblasts. Fibroblasts also contain large amounts of TF. Adipocytes secrete collagen I and III and synthesize lipids.

Platelets Platelets are the smallest of the circulating blood cells, about 2 µm in diameter. They are essential components of the haemostatic plug and are derived from bone marrow megakaryocytes. Although plate- lets are anucleate and appear to be simple cells composed of cyto- plasm, a surface connected canalicular system (SCCS), and storage granules (δ or dense granules and α-granules); they are, nevertheless, complicated cells with a variety of very important functions essen- tial for normal haemostasis. These can be broadly divided into the following: (1) platelet adhesion, defined as platelets adhering to the damaged area of the vessel wall where subendothelial matrix tissue is exposed; (2) platelet activation, both by agents within the matrix as well as by soluble agonists; (3) platelet secretion of granule contents; (4) platelet aggregation, defined as platelets sticking to one another in an aggregated mass, forming a platelet plug. The following sections describe each of these broad areas of platelet function in more detail.

Platelet adhesion The initial platelet response to vascular injury is adhesion to the vessel wall. Resting, nonactivated platelets are not attracted to the vessel wall. However, following vascular damage, platelets rolling

Table 22.7.1.5

Matrix metalloproteinases	MMP number	Activity	Substrate
MMP-1	Collagenase	Collagen I, II, III, VII, VIII, X	
MMP-2	Gelatinase	Collagen IV, V, VII, X	
MMP-3	Stromelysin	Microglycans	
MMP-8	Collagenase		
MMP-7	Matrilysin	Fibronectin, laminin, collagen IV	
MMP-9	Gelatinase	Elastin, fibronectin	
MMP-10	Stromelysin	Fibronectin, laminin, elastin, various collagens	
MMP-11	Stromelysin	Fibrinogen, fibrin	
MMP-12	Elastase	Elastin	
MMP-14	–	Collagen IV, procollagenase A	
MMP-15	–	Gelatin	

MMP, matrix metalloproteinase. Modified from Plow EF, Ugarova T, Miles LA (1998). In: Localzo J, Shafer AI, eds. Thrombosis and hemorrhage, 2nd edn, ch. 18, p. 381. Williams and Wilkins, Baltimore.

Table 22.7.1.4 The extracellular matrix

Structural proteins	Collagens I, III, IV, V, VI, VII	Elastin
Adhesive proteins	Fibronectin	Vitronectin
	Laminin	Von Willebrand factor
	Antiadhesive	Tenascin
	Thrombospondin	Ground substance
	Hyaluronic acid	Proteoglycans
	Chondroitin sulphate	Dermatan sulphate
	Heparan sulphate	Degradation and repair

Matrix metalloproteinases

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along the endothelium rapidly adhere to the subendothelial inter- stitial matrix that is exposed by injury. A number of matrix proteins, such as VWF, fibronectin, fibrinogen, and thrombospondin, are also present in platelet granules as well as in the circulating blood. Platelets possess numerous mechanisms for adhering to the subendothelial matrix (Fig. 22.7.1.3). Adhesion is accomplished by a number of protein receptors on the surface of platelets as described in the following sections.

Glycoprotein Ib-IX-V (CD42a-d)

The main function of the platelet membrane glycoprotein (GP) Ib- IX-V complex is to act as a receptor that mediates VWF-dependent binding of platelets to collagen, resulting in adhesion of platelets to the vessel wall. Glycoproteins Ibα, Ibβ, IX, and V are members of the leucine-rich glycoprotein family and are characterized by the presence of a common structural motif in the extracellular domain composed of a leucine-rich sequence. GPIbα contains seven leucine- rich repeats; GPV contains 15 leucine-rich repeats; while GPIbβ and GPIX each contain a single leucine- rich repeat, all in the extracellular domains. GPIbα, GPIbβ, GPIX, and GPV are synthesized as separate gene products which coassociate in a ratio of 2:2:2:1 during transit through the endoplasmic reticulum. Coassociation of GPIbα, GPIbβ, and GPIX, but not GPV, is required for the

complex to be expressed on the surface of cells. The role of GPV, a substrate for thrombin, in the function of the complex is uncertain, and mice deficient in GPV bind VWF normally and have normal platelet adhesion. Adhesion to VWF-coated surfaces through GPIb-IX-V is increased by shear, which is thought to induce a structural change in the receptor that enhances the interaction with VWF. The A1 domain of VWF forms the principal site that interacts with GPIb. Binding occurs in the N-terminal 45-kDa tryptic fragment from GPIb α . Within this region of GPIb α , an anionic site, 276YDYYP EE282, containing two sulphated tyrosine residues at tyrosines 278 and 279, has been further implicated in VWF binding. The A3 domain of VWF mediates the interaction with collagens type I and III. A model derived from the crystal structure of the VWF A3 domain suggests that the VWF-collagen interaction is primarily between negatively charged residues in the A3 domain and positively charged residues in collagen. Plasma VWF does not interact with unstimulated circulating platelets. For binding to occur, platelets have to be activated and plasma VWF must undergo a conformational change. After secretion by endothelial cells, VWF binds to underlying connective tissue matrix, providing an active surface for platelet attachment after the vessel wall is damaged.

Glycoprotein IIb-IIIa (α IIb- β 3) Under conditions of low shear, platelets can adhere to matrix-bound VWF through a mechanism that involves platelet glycoprotein GPIIb-IIIa (α IIb- β 3). α IIb and β 3 are members of the integrin superfamily, a conserved family of heterodimeric surface receptors, each composed of a larger two-chain α subunit and a smaller β subunit, bound noncovalently. Integrins were initially identified by an ability to bind adhesive glycoproteins containing a tripeptide sequence, arginine-glycine-aspartic acid (RGD), although subsequent work has identified other ligand sequences recognized by integrins. The interaction of VWF with α IIb- β 3 is mediated by an RGD sequence in the C4 domain of VWF. α IIb- β 3 is also able to bind fibronectin, thrombospondin, and vitronectin and may therefore represent an adhesion receptor with broad specificity.

Glycoprotein Ia-IIa (α 2 β 1) GPIa-IIa is a receptor for types I and IV collagen and mediates platelet adhesion to the vessel wall independent of VWF. The integrin sequences that mediate the interaction with collagen reside in a broad sequence called the I domain in the extracellular portion of the molecule. GPIa-IIa is constitutively active and does not require activation to interact with collagen.

Glycoprotein VI-Fc receptor γ -chain complex GPVI-Fc receptor γ -chain is the major platelet receptor mediating collagen-induced activation of platelets. GPVI is a member of the immunoglobulin superfamily and is characterized by immunoglobulin domains, a transmembrane domain, and a short cytoplasmic tail that lacks known signalling components. GPVI is associated on the platelet surface with Fc receptor γ -chain, apparently as a dimer in a 1:1 stoichiometry. The complex binds collagen and mediates collagen-generated signals through the immunoglobulin receptor tyrosine-based activation motif (ITAM) of the Fc receptor γ -chain. Cross-linking of the GPVI-Fc receptor γ -chain leads to tyrosine phosphorylation of the ITAM sequence by Src family kinases, Fyn and Lyn. Syk, another tyrosine kinase, binds to the phosphorylated ITAM sequence through its SH2 domains, initiating a signal that eventually leads to tyrosine phosphorylation of phospholipase $Cy2$ and the generation of inositol phospholipids.

Glycoprotein IV (CD36) CD36 is a highly glycosylated transmembrane protein present on platelets, monocytes, endothelial cells, and nucleated erythrocytes which binds thrombospondin. The thrombospondin-binding site has been mapped to amino acids 90 to 110 in a single disulphide loop in the extracellular domain of GPIV. Platelets deficient in GPIV have mild disturbances in platelet function and poor responses to pathological agonists such as oxidized LDL and MRP8/14.

Integrins Fibrinogen, Fibronectin, Vitronectin, vWf, Thrombospondin LRG IIb/IIIa Ia/IIa Ic/IIa VnR Ib/IX/V Ig GPVI GPVI CD36 Collagen Fibronectin Vitronectin Laminin Collagen Collagen vWf Ic/IIa Fig. 22.7.1.3

Receptors mediating the interaction of platelets with subendothelial matrix proteins. Adhesion

receptors on platelets include members of the integrin family, leucine-rich glycoproteins (LRG), members of the immunoglobulin (Ig) family, and others. Integrins on the surface of platelets are glycoproteins (GP) IIb-IIIa, which binds multiple ligands; GPIa-IIa, a collagen receptor, GPIc-IIa, which binds fibronectin; VnR, which is a receptor for vitronectin; and GPIc'-IIa, a laminin binding site. Glycoproteins Ib/IX/V are leucine-rich glycoproteins. Glycoprotein VI (GPVI)/Fc receptor γ -chain is a member of the immunoglobulin family and a collagen receptor. Glycoprotein IV (GPIV, CD36) is also a collagen receptor.

section 22 Haematological disorders 5496 Other adhesion receptors Platelets can also adhere to subendothelial matrix through their fibronectin receptor ($\alpha 5\beta 1$, glycoprotein Ic-IIa, VLA-5), lam- inin receptor ($\alpha 6\beta 1$, glycoprotein Ic'-IIa, VLA-6), or vitronectin receptor ($\alpha v\beta 3$, VnR). $\alpha 5\beta 1$ is a constitutively active receptor for fibronectin that does not require cell activation. There are two sequences in fibronectin which interact with GPIc-IIa: an RGD sequence in the tenth type III repeat which interacts primarily with the $\beta 1$ subunit and a synergy sequence in the adjacent ninth type III repeat which interacts primarily with the $\alpha 5$ subunit. $\alpha 6\beta 1$ is a lam- inin receptor which is expressed on platelets. Immunoprecipitation studies suggest that $\alpha 6\beta 1$ may exist on the cell surface in a com- plex with proteins with four transmembrane domains, so-called tetraspanins, such as CD9, CD81, and NAG-2. The nature of these interactions is presently unclear. $\alpha 6\beta 1$ recognizes a sequence in the long-arm E8 fragment of laminin obtained after elastin digestion. The binding requires the presence of divalent cations which bind to specific sites on the integrin α subunit. Small numbers of $\alpha v\beta 3$ are expressed on platelets. Current evidence indicates that all of these adhesion mechan- isms may be important. The redundancy in adhesion receptors may (1) provide backup mechanisms to protect against blood loss; (2) generate different signals in response to interaction with dif- ferent matrix proteins; or (3) represent different systems at work in different parts of the vascular tree. An example of the latter might be the relative roles of GPIb-IX-V and GPIIb-IIIa in the VWF-mediated adhesion of platelets to collagen. Under high- shear conditions, such as those found in capillaries and small arterioles, GPIb-IX-V may be the predominant mechanism mediating platelet adhesion to collagen and VWF-dependent ad- herence; whereas under low shear conditions, like those found in large veins and in arteries, GPIb-IX-V may be less effective and other mechanisms that require a shorter residence time of plate- lets on the subendothelial matrix, including GPIc-IIa interaction with fibronectin and GPIa-IIa interaction with collagen, may be important. The presence of multiple receptors for collagen on the platelet surface, including GPIb-IX-V, GPIIb-IIIa, GPIa-IIa, GPIV, and GPVI is interesting and raises the possibility of different collagen responses. Vitronectin also appears to be important for ad- hesion at high shear, and can bind to both GPIIb-IIIa and specific vitronectin receptors. Recent evidence suggests that platelet adhe- sion to collagen types I and III in flowing blood is dependent on both VWF and fibronectin. Collagen types I, II, and III have been shown to bind VWF. Platelet activation Following adhesion and in response to soluble agonists such as thrombin, platelets undergo a series of complex biochemical reac- tions leading to cell activation. As a result, platelets undergo changes in shape, alterations in surface lipid composition leading to the ex- pression of platelet coagulant activity (see later) and thrombin gen- eration, as well as secretion of the contents of intracellular granules leading to the release of ADP. The thrombin generated at the platelet surface and ADP secreted from platelet granules lead to activation of additional platelets. These reactions involve the metabolism of mem- brane inositol phospholipids, changes in cellular levels of calcium, activation of contractile proteins, stimulation of heterotrimeric and low molecular weight GTP- binding proteins, and tyrosine and serine-threonine phosphorylation of proteins, among other

events. These biochemical reactions initiate second messenger signals that drive the functional changes that occur in platelets which transform them from the resting state to an activated one, and which play a crucial role in haemostasis. Some of these signalling pathways are described in the following sections (Fig. 22.7.1.4). Phospholipid metabolism Metabolism of membrane phospholipids is one of the first signalling pathways identified in platelets and remains one of the most important. Platelet stimulation by a variety of agonists results in activation of membrane-associated phospholipases, including phospholipases C, A₂, and D, which cleave fatty acids from the phospholipid. The lipid products generated by these pathways are signalling compounds which are important for changes in cytoplasmic calcium and activation of kinases and phosphatases.

Fig. 22.7.1.4 Signalling pathways involved in platelet activation. Following the interaction of agonist with receptor, there is G protein (Gs) coupled activation of phospholipid metabolic pathways through phospholipase A₂ (PL-A₂), phospholipase C_γ (PL-C_γ), and phospholipase D (PL-D) leading to generation of thromboxane A₂ (TxA₂), inositol trisphosphate (IP₃), diacylglycerol, and phosphatidic acid (PA). Arachidonic acid generated by the action of phospholipase A₂ is converted by cyclooxygenase-1 (COX-1) to prostaglandin endoperoxides G₂ (PGG₂) and H₂ (PGH₂) which are, in turn, converted to thromboxane A₂ through the action of thromboxane synthase (TxS). Thromboxane generated through arachidonate metabolism plays a key role in secretion, perhaps through membrane fusion. Granule contents, including adenosine diphosphate, are emptied into the surface connected canalicular system (SCCS) and make their way to the outside of the cell. Diacylglycerol stimulates activation of protein kinase C (PKC), resulting in serine-threonine phosphorylation of proteins such as pleckstrin. Inositol trisphosphate (IP₃) stimulates calcium release from storage sites in the dense tubular system (dts). The release of calcium from the dense tubular system is antagonized by cyclic AMP, generated through G protein (G_{as}) coupled inhibitory receptor activation of adenylate cyclase. Calcium, released in response to IP₃, activates gelsolin, an actin-capping and -severing protein, which generates actin monomers that then serve as nucleation sites for formation of actin filaments and assembly of the activation-dependent cytoskeleton. Assembly of the cytoskeleton and interaction of the cytoskeletal proteins with surface integrins such as αIIb-β₃ (glycoproteins IIb and IIIa) are involved in integrin activation. Calcium also activates myosin light chain kinase which phosphorylates myosin light chain, generating actinomyosin contraction, important for changes in platelet shape and the secretion process.

22.7.1 The biology of haemostasis and thrombosis 5497 The most intensively studied of these pathways is the metabolism of inositol phospholipids through phospholipase C. Membrane phosphatidylinositol (PI) exists in multiple phosphorylation states: PI, PI-P, PI-P₂ which is phosphorylated in the 3,4 or 4,5 positions, and PI-P₃ which is phosphorylated in the 3,4,5 positions. Phosphatidylinositol-specific kinases and phosphatases maintain pools of phosphorylated phosphoinositides in a proper concentration range. Platelets contain several isoforms of phospholipase C which are activated by different mechanisms. All cleave phosphatidylinositol 4,5-bisphosphate (PI 4,5-P₂) and, later, phosphatidylinositol, as well as phosphatidylinositol 4-phosphate (PI 4-P), to yield diglyceride and inositol trisphosphate (IP₃). Phospholipase C_α and C_β are coupled to heterotrimeric G proteins where phospholipase C_α is coupled to growth factor receptors. IP₃ generated by phospholipase C cleavage of inositol phospholipids has been implicated in the release of calcium from intracellular storage sites in the platelet-dense tubular system. The other product of phospholipase C cleavage, diacylglycerol, activates protein kinase C, which phosphorylates pleckstrin, a 47-kDa protein, and other proteins. Phospholipase A₂ is linked to G-protein coupled receptors and cleaves fatty acids in the sn-2 position in membrane phospho-

lipids, primarily phosphatidylcholine. In most individuals in developed countries, the fatty acid in this position is arachidonic acid. Arachidonic acid, liberated by the action of phospholipase A₂, is converted to a variety of possible products by the microsomal enzymes, cyclooxygenase and lipoxygenase. Cyclooxygenase converts arachidonic acid to prostaglandin endoperoxides, prostaglandins F₂, E₂, and D₂, whose main fate in platelets is rapid conversion to thromboxane A₂ by thromboxane synthase. Thromboxane A₂ is believed to play an important role in the release of intracellular granules by acting as a membrane fusogen, fusing granule membranes with the membrane of the surface connected canalicular system and permitting secretion of the granule contents to the outside of the cell. Thromboxane A₂ is also an exceptionally potent constrictor of vascular smooth muscle and a strong platelet-aggregating agent. Inhibition of the arachidonate pathway has been a primary target for platelet inhibition. Cyclooxygenase is irreversibly inhibited by aspirin, which acetylates serine 340 of cyclooxygenase, and reversibly inhibited by nonsteroidal anti-inflammatory agents. Inhibition of cyclooxygenase inhibits thromboxane formation and results in inhibition of the release of intracellular granules. One of the mechanisms by which aspirin is thought to act as an antiatherosclerosis agent is by inhibition of the release of PDGF from platelet granules. Phospholipase D acts primarily on phosphatidylcholine to produce choline and phosphatic acid. Protein kinase C and PI-3K play an important role in activation of phospholipase D. Phosphatidic acid is an intracellular messenger which is proposed to play a role in platelet activation. In addition, phosphatidic acid can be converted to lysophosphatidic acid through the action of phospholipase A₂. Like phosphatidic acid, lysophosphatidic acid is an intracellular messenger which is involved in phospholipase activation, signalling by low molecular weight G proteins, and cytoskeleton reorganization.

Calcium metabolism Calcium ions are extremely important in platelet function, as described in subsequent discussions. In resting platelets, the cytoplasmic concentration of calcium is maintained at a low level by active transport of calcium both inside and outside the cell and into the dense tubular system (DTS), a sarcoplasmic reticulum-like fraction in platelets. Calcium transport in the platelet is accomplished by a plasma membrane sarcoplasmic-endoplasmic reticulum-like calcium ATPase (SERCA2-b), a dense tubular system SERCA3, a sodium-calcium exchange pump in the plasma membrane, and passive calcium fluxes. During platelet activation, IP₃, generated by metabolism of membrane inositol phospholipids, induces the rapid release of calcium stored in the dense tubular system. This increase in cytoplasmic calcium is essential for platelet activation, and agents that cause decreases in cytoplasmic calcium inhibit platelet activation while agents that increase cytoplasmic calcium stimulate platelet activation. Calcium functions as a major intracellular messenger in platelets, mediating calcium-dependent reactions important in almost all phases of platelet activation. An increase in the concentration of cytoplasmic free calcium activates gelsolin, the calcium-dependent actin capping and severing protein, which plays an important role in reorganization of the cytoskeleton. Calcium also activates the calcium and calmodulin-dependent myosin light chain kinase, leading to phosphorylation of myosin light chains, activation of actin-stimulated myosin ATPase activity, and the development of contractile forces. The contraction generated by actin and myosin mediates changes in platelet shape and is important for events leading to platelet secretion. In the absence of calcium ions, tropomyosin inhibits the interaction of myosin with actin, and this may be an additional regulatory role of calcium in platelets. Calpain, a calcium-dependent thiol protease, hydrolyses numerous proteins involved in platelet signalling. Activation of calpain is believed to be important both for regulation of cytoskeletal events and integrin-mediated signalling.

Cytoskeletal reorganization Resting platelets are discoid in shape and feature a cellular cytoskeleton that consists of a network of actin filaments that fill and shape the

cytoplasm of the cell and a single microtubule coil at the margin of the disc. Upon activation, platelets undergo remarkable morphological changes (Fig. 22.7.1.5). There is an initial change from the normal discoid shape of the resting platelet to a sphere as calcium levels in the cell increase. Filamentous actin appears in the form of stress fibres, and the cellular content of filamentous actin increases. Membrane ruffles form as long cellular projections called pseudopodia, processes that also involve the low molecular weight GTPases rac, rho, and cdc 42. Actin cables are present in these pseudopodia, extending to the end of the projections. Also during activation, microtubules contract and 'squeeze' granules toward the centre of the cell. The energy for contraction is provided by a magnesium ion- dependent ATPase present in myosin and stimulated by actin. Contraction occurs by actin filaments and myosin rods sliding over one another. Myosin light-chain phosphatase dephosphorylates and inhibits myosin. Membrane glycoproteins GPIIb-IIIa, GPIb-IX-V, and other membrane proteins are associated with the cytoskeleton and provide direction for the contractile process. This activation- dependent cytoskeleton is more than just a structural scaffold for platelet shape changes. Numerous signalling proteins are incorporated into the cytoskeleton and may function in specialized compartments by virtue of their association with the cytoskeleton.

section 22 Haematological disorders 5498 Platelet coagulant activity (platelet factor 3) Platelet membranes have an asymmetrical distribution of phospholipids, with almost all of the acidic (negatively charged) phospholipids such as phosphatidylserine and phosphatidylinositol located in the inner leaflet of the plasma membrane in resting platelets. After platelet activation, the acidic phospholipids are translocated to the outer half of the membrane, while phosphatidylcholine moves to the inner half, through the action of TMEM16F, a membrane-bound, calcium-dependent lipid scramblase. The exposed phosphatidylserine and other negatively charged phospholipids account for some of the activity traditionally known as platelet factor 3 by contributing to surface properties for binding of factor X and prothrombin activation complexes. This interaction with platelet phospholipids increases the rate of factor X activation and prothrombin activation nearly a thousand fold. In addition to phospholipids on the platelet membrane, there appear to be other specific binding proteins for blood clotting factors. cAMP pathway A major mechanism for down-regulation of platelet function is the stimulation of adenylate cyclase, which increases cAMP concentrations. Adenylate cyclase is mainly localized in microsomal fractions and is stimulated by adenosine, prostacyclin, and prostaglandin E1 through activation of Gs, a heterotrimeric GTPase associated with the prostaglandin receptor on the platelet surface. cAMP inhibits platelet aggregation, platelet secretion, and platelet adhesion to the vessel wall. These effects are probably exerted by inhibiting calcium flux and/or promoting calcium reuptake. Activation by soluble agonists In addition to activation through interaction with subendothelial connective tissues, platelets may also be activated by soluble agonists. These include ADP, adrenaline, and thrombin. In general, this activation occurs through the interaction between soluble agonist and specific receptors on the platelet surface. Thrombin is one of the most powerful of platelet agonists. Generated during blood coagulation, thrombin activation of platelets occurs through a novel family of receptors called PARs. These are G protein-coupled, seven-membrane-spanning molecules which are activated by proteolysis. Thrombin cleaves the N-terminal exodomain, unmasking a new N-terminal, which functions as a tethered peptide agonist. The tethered peptide binds intramolecularly to the remainder of the receptor to trigger activation. Four members of the PAR family of receptors have been identified, but only PAR-1 and PAR-4 mediate activation of human platelets by thrombin. Thrombin interacts with other proteins on the surface of platelets,

but the nature of these interactions is uncertain. Glycoprotein V, part of the GPIb-IX-V complex, is a substrate for thrombin although the absence of GPV does not appear to inhibit thrombin activation of platelets. GPIb is an equilibrium binding site for thrombin. Patients with a deficiency of GPIb have been reported to have changes in the rate of activation of platelets by thrombin which is overcome at higher concentrations of agonist. There are at least three receptors for ADP on platelets, all members of the seven-transmembrane-spanning members of the purinergic (P2) receptor family, either P2Y (G-protein-coupled purinergic receptors) or P2X (ligand-gated channel receptors). One receptor, designated P2Y₁, is coupled to phospholipase C β , probably through G_q. A second receptor, P2Y₁₂, is coupled to adenylate cyclase through G_{i2}. The third receptor, P2X₁, is coupled to rapid calcium influx and is a member of the intrinsic ion channel family. Full platelet activation by ADP likely involves an interaction of ADP with all three receptors. ADP-induced activation of GPIIb-IIIa on platelets

Microtubules Surface-connected canalicular system Alpha granule Dense granule Glycogen Mitochondrion (b) (a) Fig. 22.7.1.5 Platelet morphology. Platelets are small, anucleate cells. In the resting state (a), platelets are discoid shaped and contain a marginal rim of microtubules. After activation (b), platelets undergo changes in shape, becoming more rounded, and extend cytoplasmic projections, called pseudopods, outward.

22.7.1 The biology of haemostasis and thrombosis 5499 requires both P2Y₁ and P2Y₁₂ and concomitant signalling through the GTP-binding proteins G_q and G_{i2}. Platelet secretion A primary endpoint of platelet activation is the secretion of platelet granule contents to the outside of the cell. During platelet activation, the granules are 'squeezed' to the centre of the cell where the granules fuse with the surface-connected canalicular system, a series of intracellular canals that are connected to the cell surface. The contents of the granules make their way to the outside of the cell. Secretion requires prostaglandin metabolism and is dependent on contractile events and members of the soluble N-ethylmaleimide sensitive factor attachment protein receptors (SNAREs) which mediate granule tethering and docking with the plasma membrane. Products of prostaglandin metabolism, primarily thromboxane A₂, may act in the fusion of the granule membrane with that of the surface-connected canalicular system. Platelets possess two types of storage granules (Table 22.7.1.6), both of which are involved in secretion of active ingredients that modulate platelet function. One type is the dense granule, so called because it is dense when viewed by electron microscopy. The other type is the α -granule. Dense granules contain adenine nucleotides, calcium, and serotonin. Adenine nucleotides are sequestered in the dense granules mainly as ADP and ATP in a complex with calcium ions and pyrophosphate, and are not interchangeable with the nucleotides involved in general cell metabolism. ADP released from platelet-dense granules activates additional platelets and recruits them to the growing platelet thrombus. Serotonin, a potent modulator of vascular tone and integrity, is also a constituent of dense granules. α -Granules contain PDGF, β -thromboglobulin, PF₄, fibrinogen, factor V, VWF, and thrombospondin. PDGF is mitogenic for smooth muscle cells and when released from platelets at a site where the vessel wall is damaged, it stimulates proliferation and migration of smooth muscle cells in the intima, contributing to the atherosclerotic process. β -Thromboglobulin and PF₄ are basic, lysine-rich proteins that interact with glycosaminoglycans such as heparan sulphate, dermatan sulphate, and chondroitin sulphate, which are components of the endothelial cell surface. PF₄ has a strong heparin-neutralizing activity and has been implicated in the aetiology of heparin-induced thrombocytopenia. Thrombospondin is a major α -granule glycoprotein, but it is also secreted by fibroblasts, endothelial and smooth muscle cells. Thrombospondin is a high molecular weight adhesive protein which binds to glycosaminoglycans, fibrinogen, plasminogen, histidine-rich

glycoprotein, type V collagen, and calcium ions. It associates with cell surfaces and extracellular matrices and facilitates cell-cell and cell-matrix interactions. Platelet aggregation Platelet aggregation, the interaction of one platelet with another, is a major function of platelets and is very important in the haemostatic process. The formation of an aggregated platelet mass at the site of injury provides a physical plug that occludes the defect in the vessel wall and prevents blood loss. Aggregation is mediated by two glycoproteins on the platelet surface, α IIb- β 3, which constitute a receptor for fibrinogen/fibrin. Thus, α IIb- β 3 on one platelet binds fibrinogen or fibrin which, by virtue of its dimeric structure, interacts with α IIb- β 3 on another platelet. On resting platelets, α IIb- β 3 is in an inactive state and is unable to bind fibrinogen. Following platelet activation, α IIb- β 3 becomes activated through a process that involves calcium, protein kinase C, heterotrimeric G proteins, and talin. Activation of α IIb- β 3 requires energy and is a multistep process. Fibrinogen binding to α IIb- β 3 occurs through a C-terminal dodecapeptide sequence, HHLGGAKQAGDV (His, His, Leu, Gly, Gly, Ala, Lys, Glu, Ala, Gly, Asp, Val), in the α chain of fibrinogen where the AGDV sequence has been suggested to have structural similarity to the RGD (Arg, Gly, Asp) sequence, a common binding motif for integrins.

Blood coagulation The blood coagulation system consists of a number of zymogens (proenzymes) that are proteolytically converted to active enzymes in a series of steps involving activators and cofactors. The coagulation reactions are initiated by TF in complex with activated factor VII (VIIa). The TF-VIIa complex then activates both factor IX and factor X, which, in the presence of their respective cofactors (the activated forms of factors VIII and V), lead to the rapid conversion of prothrombin to thrombin. Thrombin converts fibrinogen into a solid fibrin clot that finally undergoes cross-linking by activated factor XIII to become a stable haemostatic plug.

Platelets are Table 22.7.1.6 Platelet granule contents α Granules α 2-Antiplasmin Immunoglobulin Albumin Multimerin β -Amyloid precursor Plasminogen activator β -Thromboglobulin (β -TG) Platelet-derived growth factor (PDGF) Clusterin Platelet factor 4 (PF4) Endothelial cell growth factor (ECGF) P-selectin (GMP-140) Factor V Transforming growth factor (TGF)- α Fibrinogen Transforming growth factor (TGF)- β 1 Fibronectin Vitronectin Granule membrane protein (GMP) 33 von Willebrand factor (VWF) Dense (δ) granules Adenosine diphosphate (ADP) Granulophysin Adenosine triphosphate (ATP) Polyphosphate (PPi) Calcium Magnesium Guanosine diphosphate (GDP) Serotonin (5-hydroxytryptamine) Guanosine triphosphate (GTP) Lysosomal (γ) granules β -Galactosidase Elastase β -Glucuronidase Endoglucosidase β -Glycerophosphatase LAMP-1 β -Hexosaminidase LAMP-2 Cathepsins LIMP-CD63 Collagenase N-acetylglucosaminidase

section 22 Haematological disorders 5500 essential in several steps of the clotting mechanism and form the surface for activated clotting factors, which lead to the explosive generation of thrombin and subsequent clot formation. Activated platelets aggregate and localize the haemostatic plug at the site of injury. The initial generation of relatively small amounts of thrombin is essential for feedback activation of factors V, VIII, XI, and XIII as well as platelets. Understanding the modern concept of the clotting reactions requires a detailed knowledge of each of the clotting factors. Table 22.7.1.7 depicts the clotting factors and their inhibitors, including the vitamin K-dependent clotting proenzymes, the nonvitamin K-dependent zymogens, the cofactors, the inhibitors of the clotting factors, and the structural proteins. Vitamin K-dependent zymogens The vitamin K-dependent blood clotting zymogens include pro-thrombin, factor VII, factor IX, factor X, protein C, and protein S; their characteristics are listed in Table 22.7.1.7 and their schematic structures in Fig. 22.7.1.6. A common feature of all these clotting factors is the presence of γ -carboxyglutamic acid (Gla) domains in the N-terminal region of the molecules. Glutamic acid residues in these proteins undergo carboxylation, a post-translational event

Table 22.7.1.7 Characteristics of coagulation

proteins Protein Plasma concentration (µg/ml) Biological half-life (h) Chromosome Vitamin K-dependent zymogens Prothrombin 100–150 60–70 11p11–q12 Factor VII 0.5 3–6 13q34 Factor IX 4–5 18–24 Xq27.1–q27.2 Factor X 8–10 30–40 13q34 Protein C 4–5 6 2q13–q14 Nonvitamin K-dependent zymogens Factor XI 5 72 4q32–q35 Factor XII 30 60 5q33 Prekallikrein 50 35 4q35 Factor XIII-A chain^a,^b 10 240 6p24–p25 Soluble cofactors Factor V^b 5–10 12 1q21–q25 Factor VIII 0.1–0.2 8–12 Xq28 Von Willebrand factor 10 12 12p13.2 Protein Sc 25 42 3p11.1–q11.2 Protein Z 2.9 ? 13q34 High molecular weight kininogen 70 150 3q26 Factor XIII-B chain^a 1q31–q32.1 Cellular cofactors Tissue factor – – 1p21–p22 Thrombomodulin – – 20p12–cen Structural protein Fibrinogen 2000–4000 72–120 α chain 4q23–32 β chain 4q23–q32 γ chain 4q23–q32 Inhibitors Antithrombin 150–400 72 1q23–q25 Tissue factor pathway inhibitor 0.1 2q31–q32.1 Protein Z-dependent protease inhibitor (ZPI) ^a All of the plasma factor XIII-A chain is in complex with factor XIII-B chain; only half of factor XIII-B chain is in complex with factor XIII-A chain, the rest is free in plasma. ^b Platelets carry significant amounts of factor XIII^a (roughly half of the total factor XIII activity) and factor V (20% of circulating factor V). The B chain of factor XIII is not in platelets. ^c Some protein S is in complex with C4b binding protein. Reprinted by permission of McGraw-Hill Companies from Roberts HR et al. (2001). Molecular biology and biochemistry of the coagulation factors. Williams Hematology, 6th edn, p.1460.

22.7.1 The biology of haemostasis and thrombosis 5501 that is affected by hepatic carboxylase that requires the reduced form of vitamin K as a cofactor. The vitamin K-dependent factors are highly homologous in terms of amino acid sequence. Factors VII, IX, X, and protein C have a similar domain structure with a Gla domain, two EGF domains, and a catalytic domain (Fig. 22.7.1.6). Prothrombin differs from other vitamin K-dependent factors in that it has two kringle domains (Fig. 22.7.1.6). Both factor X and protein C are secreted as two-chain zymogens while the others are secreted as single-chain proteins. The Gla domains of these factors are necessary for binding to phospholipid membranes, such as the surface of activated platelets. Calcium ions occupy the Gla domain to result in a conformational change in the protein that favours binding to platelet membrane surfaces. Phosphatidylserine is the major phospholipid in these reactions. The vitamin K zymogens are all serine proteases with the typical active site: a serine/histidine/aspartic acid triad. Exposure of the active site requires that the zymogen be activated by cleavage of specific arginyl residues. As a result, all the activated vitamin K-dependent zymogens become two-chain enzymes linked by disulphide bonds as depicted in Fig. 22.7.1.6. Despite the high degree of sequence homology of these proteins, they are highly specific in their interaction with their cofactors and substrates.

Prothrombin/thrombin Prothrombin is synthesized in the liver and has a molecular mass of about 72 kDa. The molecule has 10 Gla residues that play a role in the binding of prothrombin to the surface of activated platelets where it is converted to the active enzyme, thrombin, by the so-called prothrombinase complex consisting of factors Xa/Va/Ca²⁺ on the platelet surface. Thrombin is a potent enzyme with a molecular mass of about 38 kDa that rapidly converts fibrinogen to a fibrin clot. Thrombin also has many other actions including its role as a potent activator of platelets; an activator of smooth muscle cells; an activator of factor V, VIII, and XIII; an activator of protein C in the presence of its cofactor thrombomodulin; an activator of procarboxypeptidase to form thrombin-activatable fibrinolysis inhibitor (TAFI); and as a growth factor. The primary inhibitor of thrombin is AT. Factor VII Factor VII is synthesized in the liver and has a molecular mass of about 50 kDa. It has a very short half-life of 3.5 h. The specific

Prothrombin Pre-pro leader GLA domain Catalytic domain B chain Factor VII Growth factor domains Factor IX Protein C Factor X Arg169-Leu Growth factor domains Activation peptide Activation

peptide Pre-pro leader GLA domain Growth factor domains Growth factor domains Kringle domains Catalytic domain Catalytic domain Pre-pro leader Pre-pro GLA domain leader GLA domain Pre-pro leader GLA domain Catalytic domain Activation peptide Catalytic domain Arg180-Val Arg145-Ala Arg152-Ile Arg 320-Ile Arg271-Thr Arg194-Ile Fig. 22.7.1.6 Schematic diagram of the vitamin K-dependent factors, prothrombin and factors VII, IX, X, and protein C. •, γ -carboxyglutamic acid residues; ♦, active site triad of serine, histidine, and aspartic acid; arrows denote cleavage site.

section 22 Haematological disorders 5502 receptor (and cofactor) for factor VIIa is TF, found on the surface of many cells such as pericytes that surround small vessels, fibroblasts, activated monocytes, and many other cell types. The EPCR is also a specific receptor for factor VII. Once bound to TF, factor VII must be activated for the complex to be functional. The physiological activator of factor VII is unknown, although it has been suggested that it might be activated factor X. The factor VIIa-TF complex activates both factors IX and X. The factor VIIa-TF-Xa complex is inhibited by tissue factor pathway inhibitor (TFPI). Factor VIIa is not appreciably inhibited by AT except in the presence of heparin. Factor IX Factor IX is synthesized by hepatocytes and has a molecular mass of about 57 kDa. Its plasma half-life is 18 to 24 h. The molecule has 12 Gla residues. About 40% of the factor IX molecules carry a β -hydroxyaspartic acid at position 64 of the molecule. Factor IX is activated by factor VIIa-TF and by activated factor XI, both of which cleave an arginyl bond at position 145 and 180 of the molecule to release an activation peptide of about 10 kDa. Factor IXa, in complex with its cofactor (activated factor VIII), cleaves factor X to Xa. AT will inhibit factor IXa, but the inhibition is not as rapid as the AT inhibition of thrombin or factor Xa. Factor X Factor X is also synthesized by hepatocytes and has a molecular mass of 59 kDa. It is secreted as a two-chain molecule linked by disulfide bonds and has 11 Gla residues. When activated by factor IXa or factor VIIa-TF, an activation peptide is cleaved from the heavy chain to expose the active site serine on the heavy chain. Factor Xa, in the presence of its cofactor (factor Va), rapidly converts pro-thrombin to thrombin on the activated platelet surface. The primary inhibitor of factor Xa is AT. TFPI also inhibits factor Xa. Protein C Unlike the other vitamin K-dependent zymogens, protein C is not a procoagulant, but, when activated by the thrombin-thrombomodulin complex on the surface of endothelial cells, it becomes an anticoagulant by proteolysis of factors Va and VIIIa, thus inhibiting coagulation. To function in this way as an anticoagulant, activated protein C (APC) requires a nonenzymatic cofactor, protein S, which also contains vitamin K-dependent Gla residues. Protein C is synthesized in the liver and has a very short half-life of about 6 h. It contains nine Gla residues and has a molecular mass of 59 kDa. The primary inhibitor of APC is the protein C inhibitor (PCI). Nonvitamin-K-dependent zymogens Factor XI Factor XI is synthesized in the liver as a dimeric protein composed of identical subunits. It has a molecular mass of 160 kDa and a plasma half-life of about 72 h (Table 22.7.1.7). In plasma, factor XI circulates in complex with high molecular weight kininogen (HK), a nonenzymatic cofactor. The physiological activator of factor XI is thought to be thrombin, although in vitro, it can also be activated by factor XIIa. The main function of factor XIa is to boost thrombin generation by activating factor IX on the surface of platelets, over and above the factor IX activated by the VIIa-TF complex. A few patients with factor XI deficiency have virtually no bleeding tendency, and those who do usually exhibit mild bleeding when compared to severely affected haemophilic patients. Factor XII and prekallikrein These factors have been collectively referred to as contact factors since it appears that activation of factor XII is enhanced by contact with a surface. Factor XII and prekallikrein (PK) are zymogens, which, when activated, expose an active site serine. HK is a nonenzymatic protein cofactor that circulates in complex with factor XI and PK. All of these

factors are synthesized in the liver. Unlike the vitamin K-dependent proteins, factors XI, XII, and prekallikrein all possess so-called 'apple domains' that have specific functional characteristics. Deficiencies of factor XII and PK are not associated with bleeding tendencies in patients, even with complete deficiency. However, deficiency of each factor is associated with a marked prolongation of the partial thromboplastin time. In this test and in the presence of glass, ellagic acid, or some inert earth material, factor XII is activated and in the active conformation it can activate factor XI. Factor XII, PK, and HK may not play a major physiological role in haemostasis, but there is evidence that they participate in inflammatory responses that involve blood coagulation, fibrinolysis, and kinin generation. The precise role of factor XII in coagulation reactions *in vivo* is not known. Despite the fact that patients with factor XII deficiency do not exhibit bleeding symptoms, the factor is considered to be part of the 'intrinsic system' of coagulation and in some instances it may contribute to haemostasis by virtue of exposure to collagen or other surfaces. Studies in animals lacking factor XII suggest that the protease may play a role in formation of pathological thrombi.

Factor XIII Factor XIII is a proenzyme that circulates in the plasma as a heterotetramer composed of two A chains and two B chains. Factor XIII has a molecular mass of 320 kDa and a half-life of about 10 days. It circulates in plasma in association with fibrinogen. The A chain contains the active site cysteine, while the B chain is enzymatically inactive and serves as a carrier for the A chain. The A chain is found in platelets where it is not associated with the B chain. Upon activation by thrombin, the A and B chains are separated. In addition, thrombin cleaves the A chain so as to expose the active site cysteine. The activated A chain then cross-links the α and γ chains of fibrin to form a stable, impermeable fibrin clot that is more resistant to lysis by plasmin than noncross-linked fibrin.

Cofactors Some of the cofactors are soluble and exist in circulation, namely protein S, protein Z, factors V and VIII, HK, and VWF. Others are cell bound, such as TF and thrombomodulin (Table 22.7.1.7).

Protein S Protein S is synthesized in the liver and endothelial cells and is dependent on vitamin K for complete synthesis. It circulates in plasma and is also found in platelets. It has a molecular mass of 75 kDa and a plasma half-life of about 42 h. It contains 11 Gla residues in the N-terminal region. In structure, protein S differs dramatically from the other vitamin K clotting factors in that the C-terminal end is homologous to growth hormone. Protein S acts as a cofactor

22.7.1 The biology of haemostasis and thrombosis 5503 for activated protein C. Protein S exists in two forms: one form is bound to C4b-binding protein and the other exists as a free form in the circulation and is in equilibrium with the bound form. It is the free form of protein S that acts as a cofactor for protein C.

Protein Z Protein Z is synthesized in the liver and has a molecular mass of 62 kDa. Protein Z functions as an inhibitor of factor Xa. When protein Z is incubated with factor Xa, the activity of the latter is reduced. The inhibition of factor Xa activity is due to the presence of a protease inhibitor that requires protein Z as a cofactor. Whether protein Z has other functions is unknown.

Factor V Factor V is synthesized in the liver and has a biological half-life of between 12 and 36 h. It is a large glycoprotein with a molecular mass of 330 kDa. Factor V is highly homologous to factor VIII, and is composed of A, B, and C domains. A schematic diagram of the structure is shown in Fig. 22.7.1.7. The A domains are homologous to the copper-binding protein caeruloplasmin, so it is not surprising that this domain of factor V is involved in binding to calcium and copper. The C domains are homologous to fat-globule proteins and are involved in the binding of factor V to phospholipid-rich platelet membranes. The A and C domains are homologous to similar domains in factor VIII, but the B domain is completely different from that of factor VIII. For factor V to act as a cofactor for factor Xa, it must be activated by thrombin with cleavage of arginyl bonds at positions 708, 1018, and 1545 as shown in Fig. 22.7.1.7. It is inactivated by APC, which

cleaves bonds at 306 (slow) and 506 (fast). Factor VIII Like factor V, factor VIII is synthesized in the liver. Factor VIII mRNA is found largely in sinusoidal endothelial cells and Kupffer cells, although there is significant extrahepatic synthesis of factor VIII in endothelial cells. It is a large glycoprotein, similar in size to factor V. Again, like factor V, factor VIII has A, B, and C domains with the A domains homologous to caeruloplasmin and the C domains homologous to fat globule proteins (Fig. 22.7.1.8). The C domains of factor VIII are essential for binding to phospholipid membranes. The B domain of factor VIII is proteolytically removed during activation. To act as a cofactor for factor IXa, factor VIII must be activated by thrombin or factor Xa. Unlike activated factor V, activated factor VIII exists as a heterotrimer composed of A1, A2, and A3-C1-C2 domains linked by calcium ions. Factor VIII circulates in a noncovalent complex with VWF and has a biological half-life of 8 to 12 h. In the complete absence of VWF, such as occurs with type III von Willebrand disease, the half-life of factor VIII is less than 1 h. When activated factor VIII is released from VWF, it binds to the surface of activated platelets where it interacts with factor IXa. von Willebrand factor VWF is synthesized by endothelial cells and stored as large and ultra-large multimer in Weibel-Palade bodies. It also circulates in plasma bound to factor VIII. It binds to glycoprotein Ib on platelets and is required for normal platelet adhesion to components of the vessel wall such as collagen. A schematic diagram of VWF is shown in Fig. 22.7.1.9. Although synthesized as a prepolyptide with A, B, C, and D domains, it is secreted into the plasma in multimeric form with molecular mass ranging from 1000 kDa to 15 000 to 20 000 kDa. Higher molecular mass forms of VWF are secreted to the abluminal surface of the endothelial cell as one component of the extracellular matrix. The higher molecular mass VWF multimers are very effective in promoting platelet adhesion. VWF is also important in platelet aggregation. A major function of VWF is to act as a carrier protein for factor VIII. Factor VIII is associated with VWF multimers of all sizes. Inactivation

Me iVa C2 2196 V Va C1 C2 C1 A3 A3 C1 C2 3 A A2 A1 Activation 709 306 506 1018 1545 B A1 A2 A2 A2 A1 Me Fig. 22.7.1.7 Schematic diagram of factor V. Factor V is activated by thrombin to factor Va. Factor Va is inactivated (iVa) by activated protein C. Activation of factor V by thrombin results in loss of the B chain and formation of a heterodimeric molecule covalently linked by metal ions (Me). Inactivation is by activated protein C that cleaves arginyl bonds at positions 306 and 506. A1 B A2 372 740 C2 C1 A3 A2 A1 Thrombin A3 C1 1689 C2 ME++ Factor VIIIa (activated) Factor VIII (unactivated) Factor VIII Fig. 22.7.1.8 Schematic representation of factor VIII. Activation by thrombin (or factor Xa) results in a heterotrimer noncovalently linked by metal ions (Me). Like factor Va, factor VIIIa is inactivated by activated protein C.

section 22 Haematological disorders 5504 High molecular weight kininogen HK circulates in plasma, and part is bound to factor XI and prekallikrein. HK is a cofactor for both of these zymogens. Deficiency of HK is not associated with a bleeding tendency, although the partial thromboplastin times of affected subjects are prolonged. Tissue factor TF is a transmembrane cell surface protein. Soluble TF circulating in plasma has been described but does not appear to be functional. TF may also be found in microparticles but again its functional significance has not been well established. It is composed of 263 amino acids and with a 219-amino acid extracellular domain, a 23-amino acid transmembrane domain, and a 21-amino acid intracytoplasmic domain. The characteristics of TF are shown in Table 22.7.1.7. It has a molecular mass of about 46 kDa and is constitutively expressed on several extravascular tissues such as fibroblasts and smooth muscle cells. It is not constitutively expressed on cells exposed to the circulating blood, but can be induced in endothelial cells by certain inflammatory cytokines and certain bacterial products such as endotoxin. It can also be induced in blood leucocytes. TF functions as a receptor for factor VII.

When factor VII binds to TF, it is rapidly converted to factor VIIa, although the precise mechanism for its activation is not clear. The VIIa-TF complex is now thought to be the main physiological initiator of blood coagulation by activating both factor IX and factor X, each of which plays a distinct role in subsequent coagulation reactions as described below. On some cells TF exists in a 'latent' form, sometimes referred to as 'encrypted TF', as suggested by the fact that TF antigen levels on cells may be higher than TF functional activity. De-encryption can be accomplished by exposure of cells to agents such as calcium ionophores and various cytokines, but the physiological mechanism by which this process takes place is not known. Thrombomodulin is a transmembrane protein synthesized by and localized to endothelial cells although it has also been found on mesothelial cells, monocytes, and squamous epithelial cells. It has a molecular mass of about 78 kDa. A chondroitin sulphate moiety is attached to thrombomodulin via a serine residue. The major characteristics of thrombomodulin are depicted in Table 22.7.1.7. It serves as a receptor on endothelial cells for thrombin. Thrombin bound to thrombomodulin undergoes a structural transformation such that it no longer activates platelets or clots fibrinogen, but rather activates protein C. The principal function of the D3 D4 D3 D4 D1 D2 D4 C1 C2 C3 C4 C5 C6 CK D3 D' D3 D4 D3 D4 D3 D4 D3 D4 D3 D4 D3 D4 D3 D4 D3 D4 A1 A2 A3 propeptide propeptide Mature VWF Furin cleavage Dimerization Multimerization Flow at pH 7.4 In plasma Intracellular Fig. 22.7.1.9 Schematic diagram of von Willebrand factor (VWF). Following synthesis, VWF is cleaved by furin into a propeptide and mature VWF protein. These remain noncovalently associated as VWF dimerizes and multimerizes in the cell. In the acidic environment of the late Golgi, factor VIII associates with VWF and the propeptide dissociates. Dimerization occurs through disulphide links in the CK domain. The formation of multimeric forms of VWF occurs through links of dimeric VWF via D3 domains. In plasma, VWF may form elongated 'strings' after attachment to the vessel wall.

22.7.1 The biology of haemostasis and thrombosis 5505 thrombomodulin-thrombin complex is to prevent the extension of the haemostatic clot past the site of a break or leak in the vessel wall and as such represents an important control mechanism to restrict the haemostatic plug precisely to the point of injury. Thus, under normal conditions, clot formation does not occur on the endothelial cell surfaces. Fibrinogen is synthesized in the liver and has a molecular mass of 340 kDa. It is a dimeric glycoprotein consisting of two sets of identical chains, the α , β , and γ chains. The synthesis of each fibrinogen chain is governed by a separate gene, as depicted in Table 22.7.1.7. The normal plasma half-life of fibrinogen is about 3 to 5 days. It is also found in the α granules of platelets as a result of endocytosis. Fibrinogen is the soluble plasma precursor of the solid fibrin clot that is necessary for haemostasis and normal wound healing. A schematic diagram of fibrinogen is shown in Fig. 22.7.1.10. It is a trinodular structure with a central E domain that includes the disulphide-linked N-termini of all six polypeptide chains. The E domain is linked to the C-terminal domains referred to as the D domains. Fibrinogen conversion to fibrin is accomplished by thrombin cleavage of two fibrinopeptides (fibrinopeptide A and fibrinopeptide B) from each of the two α and β chains, respectively, leading to the formation of the fibrin monomer. The molecular mass of each fibrinopeptide A and B is about 2500 Da. The soluble fibrin monomer then undergoes spontaneous polymerization by forming side-to-side and end-to-end interactions, resulting in protofibrils that aggregate into a visible fibrin clot composed of thicker, branched fibres. During fibrin clot formation, other proteins are occluded in the clot, including plasminogen, fibronectin, thrombospondin, and VWF. Fibrin polymerization is enhanced by calcium ions, but the polymerization process alone does not lead to a stable and impermeable fibrin clot since the

fibres are held together weakly by hydrogen bonds and electrostatic forces. A stable fibrin clot requires cross-linking of the α and γ chains of fibrin by the action of activated factor XIII. Inhibitors of the coagulation reactions (See Table 22.7.1.7.) Tissue factor pathway inhibitor TFPI is synthesized by endothelial cells. It has a molecular mass of about 34 to 40 kDa and serves to inhibit the initiation of coagulation. TFPI can inhibit factor Xa in a slow reaction and also inhibits the VIIa-TF-Xa complex. TFPI exists in two forms, TPFI α and TPFI β . The latter is directly anchored to cell surfaces via glycosylphosphatidylinositol links. It exists in the circulation in at least three pools. One is bound to plasma lipoproteins; one pool is bound to proteoglycans on the vessel wall; and one exists in platelets. The TFPI bound to proteoglycans can be released by heparin. TFPI is a Kunitz-type inhibitor that is essential for control of coagulation at the initiation phase. Antithrombin AT belongs to a family of protease inhibitors known as serpins that inhibit many proteases with a serine-active site. It is synthesized in the liver and has a plasma half-life of approximately 65 h. Its major function is to inhibit thrombin and factor Xa, although it will also inhibit the other coagulation serine proteases less well. The inhibitory action of AT is greatly enhanced by heparin, which accelerates the rate of inhibition of the serine proteases. Protein Z-dependent protease inhibitor This inhibitor inhibits factor Xa in the presence of calcium, phospholipids, and protein Z. It has a molecular mass of about 72 kDa. Like AT, it is also a member of the serpin family of serine protease inhibitors. Other inhibitors of clotting factors The major inhibitor of factor XIa is thought to be α 1-antitrypsin since it has the highest affinity for the enzyme. However, other inhibitors, namely C-1 esterase inhibitor, will also inhibit factor XIa. The other inhibitors that are of some importance in coagulation are also listed in Table 22.7.1.7. Coagulation pathways The coagulation reactions have been viewed as a sequential series of steps in which an enzymatic precursor (zymogen) clotting factor is converted to an active enzyme that in turn activates another precursor, finally ending in the rapid conversion of prothrombin to thrombin. Early models of the coagulation reactions are shown in Fig. 22.7.1.11. As can be seen, when viewed in this manner, the clotting reactions appear as a waterfall or cascade, hence the terms waterfall or cascade hypotheses. Since TF was extrinsic to the blood stream, the activation of factor X by the VIIa-TF complex was termed the extrinsic pathway. The intrinsic pathway consisted entirely of clotting factors within the circulation and, upon conversion of factor IX to IXa by factor XIa, the factor IXa-VIIIa complex could also convert factor X to Xa in the presence of phospholipids. Although this concept of coagulation was essentially correct, it did not explain why patients with factor XII deficiency had no bleeding tendency, nor why factor XI-deficient patients had only a mild bleeding tendency. It was also pointed out that defects in the intrinsic system could lead to haemorrhage in affected patients even though the extrinsic system was intact and vice versa. The demonstration that the VIIa-TF complex could activate both factor IX and factor X led several groups to conclude that the clotting reactions were, in fact, initiated by factor VIIa-TF and that separate intrinsic and extrinsic systems did not exist in vivo. Further work demonstrated that the clotting reactions leading to a haemostatic plug were controlled in large part by cell surfaces which modulated the reactions.

Fig. 22.7.1.10 Diagram of the structure of fibrinogen. The three chains, α , β , γ , are shown. The E domain occurs at the N-termini while the D domains are found at the C-termini. Arrows represent cleavage sites for fibrinopeptide A from the α chain and fibrinopeptide B from the β chain.

section 22 Haematological disorders 5506 Role of the TF-expressing cell When a blood vessel is injured or ruptured, flowing blood is exposed to TF, which is bound through its transmembrane domain and cytoplasmic tail to cells exposed as the result of injury, e.g. pericytes, fibroblasts, and

other connective tissue cells. Factor VII binds to the TF-bearing cell and is activated. In fact, it appears that the TF found in pericytes surrounding small vessels is already saturated with factor VII. As a result, the VIIa-TF complex on the TF-bearing cell activates both factor IX and factor X as shown in Fig. 22.7.1.12a. The factors Xa and IXa formed in the milieu of the TF-expressing cell play very different and distinct roles in subsequent reactions. The role of factor Xa in the milieu of the TF cell Factor Xa, in concert with its cofactor Va (which is found in the vicinity of TF cells) converts prothrombin to very small amounts of thrombin, as shown in Fig. 22.7.1.12b. This amount of thrombin, though insufficient to clot fibrinogen, can, however, act as a 'primer' of subsequent coagulation reactions to accomplish the following: activate platelets; activate more factor V; dissociate factor VIII from VWF and activate factor VIII; and activate factor XI as shown in Fig. 22.7.1.12b. Factor Xa alone and in complex with VIIa-TF is then inhibited by TFPI. The activated co-factors resulting from the priming amount of thrombin in the milieu of the TF cell then occupy binding sites on the activated platelet as shown in Fig. 22.7.1.12b. Thus, the main function of factor Xa formed as the result of the VIIa-TF complex is to furnish a priming amount of thrombin sufficient to initiate further subsequent reactions which take place on the activated platelet surface. Role of factor IXa activated by the factor VIIa-TF complex Factor IXa formed by the VIIa-TF complex on the TF-bearing cell diffuses away from the TF cell and occupies a site on the activated platelet adjacent to its co-factor VIIIa (Fig. 22.7.1.12c). This factor IXa then plays a primary role in the subsequent burst of thrombin generation on platelet surfaces as noted in the next sections. Role of the activated platelet The activated platelet mass is the primary site of thrombin generation, which is highly dependent upon the amount of factor IXa formed both by the VIIa-TF cell and factor XIa, which also occupies sites on the platelet. Factor IXa in the presence of its cofactor VIIIa then recruits more factor X from solution and activates it Intrinsic pathway XII XI IX IXa XIa PK HK XIIa HK VIIIa VIIa TF Extrinsic pathway X Xa Prothrombin Fibrin Fibrinogen Thrombin XIII XIIIa Cross-linked fibrin X Va Fig. 22.7.1.11 An earlier model of blood coagulation reactions: the cascade or waterfall hypothesis of coagulation. X VIIa Xa II IIa Va TF (a) (b) (c) TF VIIa IX IXa TFPI Xa XIa X IXa IXa Xa Va VIIIa XIa IX Activated platelet II IIa VIIIa VIIa VIIIa Activated platelet Platelet Xa IIa VIII/VWF VIII + free VWF XI XIa V II Va Va Va V TF TF Tissue factor-bearing cell Tissue factor-bearing cell Fig. 22.7.1.12 (a) Tissue factor (TF), a transmembrane protein expressed on TF-bearing cells, acts as a receptor for factor VII, which is rapidly converted to factor VIIa. The TF-VIIa complex then accomplishes two major functions: (1) activation of factor X to Xa and (2) activation of factor IX to IXa. Factor Xa activates factor V on the TF-bearing cell and the resulting Xa-Va complex converts small amounts of prothrombin to thrombin. (b) The small amount of thrombin formed in the vicinity of the TF cell acts as a 'primer' for coagulation by (1) activating platelets; (2) dissociating factor VIII from VWF and activating factor VIII; (3) activating factor V; and (4) activating factor XI. The activated platelets then adhere to the site of vascular injury and bind the cofactors, factors VIIIa and Va. Factor XIa also binds to platelets. The TF-VIIa-Xa complex is then inhibited by TFPI. (c) Factor IXa formed by the TF-VIIa complex associates with VIIIa on the platelet surface and recruits additional factor X from plasma to form factor Xa. The factor Xa then associates with its cofactor, factor Va, on the platelet surface to rapidly convert prothrombin to large amounts of thrombin sufficient to clot fibrinogen.

22.7.1 The biology of haemostasis and thrombosis 5507 on the activated platelet surface. This factor Xa in the presence of its cofactor Va then converts large amounts of prothrombin to thrombin sufficient to clot fibrinogen. All of these reactions are summarized in Fig. 22.7.1.13. The mass of aggregated platelets upon which these reactions take place is localized to the damaged

area of the vessel wall. Role of the endothelial cells, vessel wall, and inhibitors The mass of platelets interspersed with fibrin forms a plug at the site of a leak in the vessel wall where the endothelial cell mono- layer is disrupted. The question arises as how the haemostatic plug is confined to the damaged area of the vessel wall. A schematic diagram of these events is shown in Fig. 22.7.1.14. The endothelial cells express thrombomodulin, which traps thrombin to form a thrombomodulin-thrombin complex that controls the procoagulant stimulus by activating the protein C system, resulting in inactivation of both factors Va and VIIIa, all localized essentially on the endothelial cell surface. This series of events is enhanced by activated protein C on the EPCR. In addition, endothelial cells contain glycosaminoglycans, some of which inhibit thrombin via AT. AT also circulates in solution to inhibit any thrombin that escapes from the haemostatic plug. In this way the fibrin clot sealing a leak in a blood vessel wall is confined precisely to that site such that extension of the clot does not occur under normal circumstances. Ongoing coagulation in vivo It is well known that products of the coagulation reactions are found in the circulation under normal (basal) conditions. Small but definite levels of fibrinopeptides A and B can be measured in plasma. Fragment 1+2 derived from the N-terminal portion of prothrombin can also be detected after thrombin is formed. Activation peptides from several of the coagulation factors as well as complexes of activated factors with their inhibitors can also be found in the circulation. These observations strongly suggest that small leaks in blood vessels that occur during the stress and strain of everyday living are repaired by the ongoing formation of haemostatic fibrin clots. This has been termed 'basal' coagulation, a process that allows the blood to remain fluid within the vascular tree and at the same time permits small, exquisitely controlled and confined fibrin clots to plug small leaks in the vasculature without dissemination. The fibrin plug is then removed by the fibrinolytic system following the formation of new tissue.

Role of TF in microparticles It has been shown that microparticles shed from leucocytes and other cells possess procoagulant activity at least in vitro and perhaps in vivo. These particles have also been shown to possess TF but the physiological role of this TF in vivo has not been definitively demonstrated. Platelets contain pre-mRNA for TF and under certain circumstances may express TF activity but again, the physiological significance of these interesting observations has not been clearly delineated.

Fibrinolytic system The fibrinolytic system is shown schematically in Fig. 22.7.1.15. The components of the system and their characteristics are depicted in

Fibrinogen Fibrin VIIa VIII/VWF Platelet VIIIa Activated platelet VIIIa + free VWF XI XIa Va V IIa XIa Va Xa II TF TFPI Xa Tissue factor-bearing cell TF VIIIa Va V TF VIIIa X II X IX IXa Xa IIa D D E D D E D D E Fig. 22.7.1.13 The clotting reactions summarized. After thrombin formation, fibrinogen is converted to fibrin. Blood flow IIa iV TF Platelet plug TM Va Endothelial cell ATIII PC G A G APC PS Endothelial cell Subendothelium TF IIa IIa PC IIa G A G ATIII TM TF TF VIIIa APC PS iVIII Fig. 22.7.1.14 Diagram of the haemostatic plug and the control mechanisms that restrict this plug to the site of injury and prevent extension of the clot to normal endothelium. APC, activated protein C; GAG, glycosaminoglycans; IIa, thrombin; iVa, inactivated Va; PC, protein C; platelet plug = haemostatic plug iVIIIa, inactivated VIIIa; PS, protein S; TF, tissue factor; TM, thrombomodulin. Plasminogen FDP Fibrin T-PA PAI pro-MMP ECM degradation U-PA:u-PAR Plasmin MMP TIMP PAI α 2-Antiplasmin Fig. 22.7.1.15 The fibrinolytic system. Plasminogen is converted to plasmin by activators, including plasminogen activators (tPA) and urokinase (U-PA). The activators are inhibited mainly by plasminogen activator inhibitor-1 (PAI-1). Plasmin degrades fibrin and activates matrix metalloproteinases (MMPs), which degrades extracellular matrix (ECM). Plasmin is inhibited by antiplasmin. FDP, fibrin degradation product; TIMP, tissue inhibitors of metalloproteinases; U-PAR, urokinase protease- activated receptor.

section 22 Haematological disorders 5508 Table 22.7.1.8. The active enzyme in the fibrinolytic system is plasmin, which is derived from its precursor, plasminogen. Plasminogen is activated to plasmin by activators. The physiological activator is single-chain tPA, which cleaves plasminogen into two-chain plasmin. Another activator of plasminogen in vivo is single-chain urokinase, but this appears to be more important for degradation of matrix proteins. The physiological inhibitor of plasmin is α 2-antiplasmin. Plasminogen and tPA associate in the circulation with fibrinogen. When fibrinogen is converted to fibrin, free lysine residues in fibrin promote the binding and conversion of plasminogen to plasmin by tPA. Fibrin-bound plasmin is protected from the inhibitory action of antiplasmin. Thus, clots can be lysed without interference from inhibitors, yet free plasmin in the circulation will be rapidly inhibited by its inhibitor. Plasminogen Plasminogen is synthesized in the liver and has a molecular mass of about 92 kDa. It is composed of a single chain and exists in two forms in the circulation: Glu-plasminogen and Lys-plasminogen. Glu-plasminogen has an N-terminal glutamic acid and is larger than Lys-plasminogen, which is formed in the circulation by plasmin cleavage of an arginyl bond at position 78 of the Glu form, leaving lysine as the N-terminal residue. Lys-plasminogen rapidly binds to fibrin via lysine binding sites. Thus, Lys-plasminogen is in close proximity to fibrin and protected from the action of antiplasmin. When activated, plasminogen is converted to active two-chain plasmin with a serine-active site on the heavy chain that is connected to the light chain by disulphide bonds. The proteolytic action of plasmin is usually characterized by the proteolysis of fibrinogen and fibrin, but it can also degrade several other proteins including factor VIII, factor V, VWF, and others. The cleavage of fibrinogen and fibrin leads to the formation of fibrin(ogen) degradation products (FDP). Fibrin(ogen) fragments resulting from plasmin cleavage are shown in Fig. 22.7.1.16. Fragment X is the first and largest fragment of plasmin digestion of fibrinogen. It is still clottable by thrombin, although much more slowly than native fibrinogen. Fragment X gives rise to fragments Y and D, and fragment Y is further proteolysed to give rise to a second fragment D plus fragment E. These fragments can be detected in a simple laboratory test using antifibrinogen antibodies coated to latex particles. However, the test is nonspecific and does not distinguish between the fibrinogen or fibrin degradation products which are quite similar, since the only difference between fibrin and fibrinogen is the absence of the small fibrinopeptides A and B in fibrin. A better test for detection of fibrin fragments is the so-called D-dimer test, which detects D-dimers resulting from the cross-linking of fibrin by factor XIIIa.

tPA tPA is considered to be the physiological activator of plasminogen. It is synthesized in endothelial cells and has a molecular mass of about 68 kDa. It has high affinity for plasminogen. tPA circulates for the most part in complex with its inhibitor, plasminogen activator inhibitor-1 (PAI-1). The tPA-PAI-1 complex can be dissociated during the process of coagulation, and free tPA associates with fibrin, which enhances tPA activity. Single-chain tPA has catalytic activity, but when activated to the two-chain form by plasmin, the activity is increased by threefold. Plasminogen activator inhibitor-1 PAI-1 is the physiological inhibitor of tPA. It belongs to the serpin family of inhibitors. It is synthesized in endothelial cells and has a molecular mass of 52 kDa. Elevated levels of this inhibitor have been associated with arterial and venous thromboses. PAI-2, found in the placenta, also inhibits tPA, but not as efficiently as PAI-1. PAI-3 is also known as the protein C inhibitor and inhibits plasminogen activators less efficiently than PAI-1. Urokinase plasminogen activator Urokinase plasminogen activator (U-PA) exists as a single-chain zymogen and is found in the kidney, the urine, and fibroblast-like cells. It activates plasminogen by proteolysis of an arginyl residue at position 561. Its main function is in wound healing and vasculogenesis, and it is active in proteolysis of the extracellular

Table 22.7.1.8 Characteristics of the fibrinolytic system

Plasma	molecular mass (kDa)	Concentration (mg/litre)	Chromosomal
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location Plasminogen 92 20 6 Plasmin 85 – tPA 68 0.005 8 U-PA 54 0.008 10 α 2-Antiplasmin 70 200
18 PAI-1 52 70 7 PAI-2 47, 60 0.0518 18 u-PAR 50, 60 Fragment X Fragment Y Fragment D
Fragment E Fragment D

- B β 1-42, A α fragments Fibrin(ogen) D E D Fig. 22.7.1.16 Plasmin digestion of fibrin(ogen) results in fibrin degradation products: X, Y, D, and E. The final protolytic fragments resulting from plasmin degradation of fibrin are two molecules of fragment D and one of E.

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