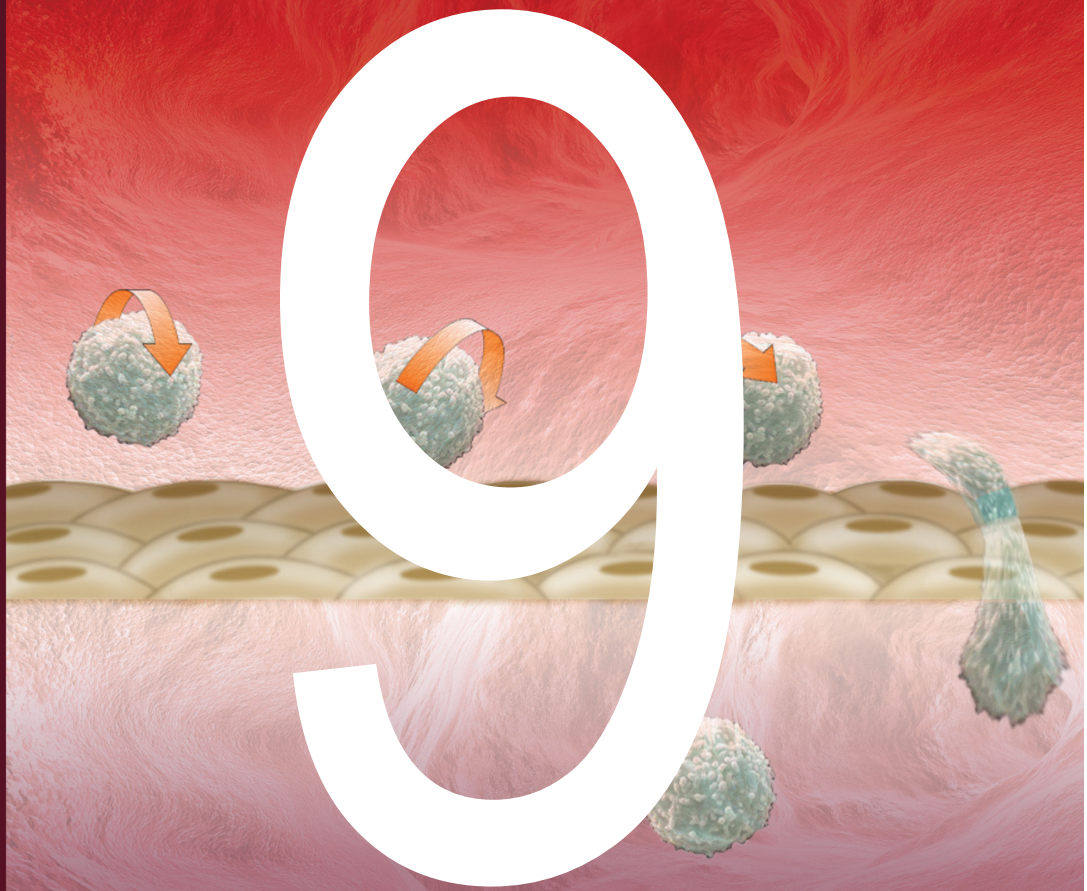
Mechanisms of Vascular Disease:

A Reference Book for Vascular Specialists



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Mechanisms of Vascular Disease

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A Reference Book for Vascular Specialists

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Abbreviation List

a1-PI	a1-protease inhibitor
5-HT	5-Hydroxytryptamine/Serotonin
AAA	Abdominal aortic aneurysm
AAS	Acute aortic syndrome
AAV	Adeno-associated viruses
ACE	Angiotensin converting enzyme
ACS	Acute coronary syndrome
ACS	Abdominal compartment syndrome
ACTH	Adrenocorticotropic hormone
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
ADP	Adenosine diphosphate
AIDS	Acquired immune deficiency syndrome
ALI	Acute lung injury
AMP	Adenosine monophosphate
AMPA	α-amino-3 hydroxy-5-methylisoxazole
ANA	Anti-nuclear antibody
ANCA	Anti-neutrophil cytoplasmic antibody
AOD	Aortic occlusive disease
AP1	Activated protein 1
APC	Activated protein C
APC	Antigen presenting cell
APLAS	Antiphospholipid antibody syndrome
ApoAI	Apolipoprotein AI
ApoE	Apolipoprotein E
APS	Antiphospholipid antibody syndrome
APTT	Activated partial thromboplastin time

ARDS	Acute respiratory distress syndrome
AT	Antithrombin
ATP	Adenosine triphosphate
AVP	Ambulatory venous thrombosis
β2-GPI	β2-glycoprotein Ib
bFGF	Basic fibroblast growth factor
BKCa	Large conductance calcium activated potassium channel
BMPs	Bone morphogenetic proteins
BMS	Bare metal stent
CAD	Coronary artery disease
CaM	Calmodulin
CAM	Cell adhesion molecule
cAMP	Cyclic adenosine monophosphate
CCK	Cholecystokinin
cGMP	Cyclic guanine monophosphate
CD	Cluster of differentiation
CD40L	Cluster of differentiation 40 ligand
CEA	Carotid endarterectomy
CETP	Cholesteryl ester transfer protein
CFD	Computational fluid dynamics
CG	Cationized gelatin
CGRP	Calcitonic gene regulated peptide
CHD	Coronary heart disease
CI	Confidence interval
CIMT	Carotid intimal-media thickness
c-JNK	c-Jun N-terminal kinase
CK-MB	Creatinine kinase (Myocardial specific)
CNCP	Chronic noncancer pain
cNOS	Constitutive nitric oxygen synthase enzyme
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CROW	Charcot restraint orthotic walker
CRRT	Continuous renal replacement therapy

CRP	C-reactive protein
CRPS	Complex regional pain syndromes
CT	Computational tomography
CTA	Computed tomographic angiography
CTD	Connective tissue disorders
CTGF	Connective tissue growth factor
CYP	Cytochrome P450
CVD	Cardiovascular disease
CVI	Chronic venous insufficiency
DAG	Diacylglycerol
DES	Drug-eluting stent
DRG	Dorsal root ganglion
DNA	Deoxyribonucleic acid
DSA	Digital subtraction arteriography
DTS	Dense tubular system
DVT	Deep vein thrombosis
EC	Endothelial cell
ECM	Extracellular matrix
EDCF	Endothelium-derived contracting factor
EDH	Endothelium-dependent hyperpolarisation
EDS	Ehlers-Danlos syndrome
EET	Epoxyeicosatrienoic acids
ELAM-1	Endothelial-leukocyte adhesion molecule-1
ELG	Endoluminal grafts
ELISA	Enzyme linked immunosorbent assay
E_K	Equilibrium potential
E_M	Membrane potential
eNOS	Endothelial nitric oxide synthase enzyme
EPC	Endothelial progenitor cells
EPCR	Endothelial protein C receptor
ePTFE	Expanded polytetrafluoroethylene
ERK	Extracellular signal-regulated kinase
ESR	Erythrocyte sedimentation rate

ET	Essential thrombocytosis
ET-1	Endothelin 1
EVAR	Endovascular aortic aneurysm repair
EVLA	Endovenous LASER ablation
FDA	Food and drug administration
FDPs	Fibrin degradation products (soluble)
FGF	Fibroblast growth factor
FGF-2	Fibroblast growth factor 2
FMN	Flavin mononucleotide
FVL	Factor V Leiden
GABA	Gamma-aminobutyric acid
GABA B	Gamma-aminobutyric acid subtype B
G-CSF	Granulocyte colony stimulating factor
GMCSF	Granulocyte-macrophage colony stimulating factor
GP	Glycoprotein
GPCR	G-protein coupled receptor
GSV	Great saphenous vein
HDL	High density lipoprotein
HDL-C	High density lipoprotein cholesterol
HIF	Hypoxia inducible factor
HIT	Heparin induced thrombocytopenia
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HMG Co-A	Hydroxymethylglutaryl coenzyme-A
HMW	High molecular weight
HPETE	Hydroperoxyeicosatetraenoic acid
HETE	Hydroxyeicosatetraenoic acids
HR	Hazard ratio
hsCRP	High-sensitive C-reactive protein
HSP	Heat shock protein
HUV	Human umbilical vein
IAH	Intra-abdominal hypertension

IAP	Intra-abdominal pressure
IAPP	Intra-abdominal perfusion pressure
ICAM-1	Inter-cellular adhesion molecule-1
ICAM-2	Inter-cellular adhesion molecule-2
ICP	Intra-compartmental pressure
ICU	Intensive care unit
IFN	Interferon
IGF-1	Insulin-like growth factor-1
IHD	Ischemic heart disease
IL	Interleukin
IL-1	Interleukin-1
IL-1α	Interleukin-1 alpha
IL1-β	Interleukin-1 beta
IL-6	Interleukin-6
IL-8	Interleukin-8
ILT	Intraluminal thrombus
IKCa	Intermediate conductance calcium-activated potassium channels
IMH	Intramural haematoma
IMP	Inosine monophosphate
iNOS	Inducible nitric oxide synthase enzyme
IP(3)	1,4,5-inositol triphosphate
IRI	Ischemia reperfusion injury
IVIG	Intravenous pooled immunoglobulin
IVUS	Intravascular ultrasound
KGF	Keratinocyte growth factor
KGF-2	Keratinocyte growth factor-2
LAP	Latency associated peptide
LCS	Limb compartment syndrome
LDL	Low density lipoprotein
LDS	Loeys-Dietz syndrome
LLC	Large latent complex
LEC	Lymphatic endothelial cells

LFA-1	Lymphocyte function-associated antigen-1
LO	Lipoxygenase
LOX	Lysyl oxidase
LOPS	Loss of protective sensation
LPA	Lysophosphatidic acid
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
LTGFBP	Latent TGF binding protein
MAC-1	Macrophage-1 antigen
MAPK	Mitogen activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
M-CSF	Macrophage-colony stimulating factor
MFS	Marfan syndrome
MHC	Major histocompatibility
MI	Myocardial infarction
MIP-1	Macrophage inflammatory protein-1
MLC_{20}	Myosin light $chain_{20}$
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
MMP	Matrix metalloproteinase
MODS	Multiple organ dysfunction syndrome
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSE	Methicillin resistant *Staphylococcus epidermidis*
MRTA	Magnetic resonance tomographic angiography
MTHFR	Methylenetetrahydrofolate reductase
MT-MMP	Membrane-type MMP
MVPS	Mitral valve prolapse syndrome
NADPH	Nicotinamide adenine dinucleotide phosphate
NGF	Nerve growth factor

NFκB	Nuclear factor kappa B
NiTi	Nitinol
NJP	Non-junctional perforators
NMDA	N-methyl-D-aspartate
NNH	Number needed to harm
NNT	Number needed to treat
NO	Nitric oxide
NOS	Nitric oxide synthase enzyme
NSAID	Non-steroidal anti-inflammatory drug
NV	Neovascularisation
OCP	Oestrogen/progesterone contraceptive pill
OPN	Osteopontin
OPG	Osteoprotegerin
OR	Odds ratio
OxLDL	Oxidised low density lipoprotein
PAD	Peripheral arterial disease
PAF	Platelet activating factor
PAI	Plasminogen activator inhibitor
PAI-1	Plasminogen activator inhibitor-1
PAR	Protease activated receptor
PAR-1	Protease activated receptor-1
PAR-4	Protease activated receptor-4
PAU	Penetrating aortic ulcer
PC	Protein C
PCA	Poly (carbonate-urea) urethane
PCI	Percutaneous coronary intervention (angioplasty)
PCWP	Pulmonary capillary wedge pressure
PDGF	Platelet-derived growth factor
PDGFβ	Platelet-derived growth factor-β
PDS	Polydioxanone
PECAM-1	Platelet-endothelial cell adhesion molecule-1
PEDF	Pigment epithelium-derived factor
PES	Paclitaxel-eluting stent

PET	Positron emission tomography
PF4	Platelet factor 4
PGI_2	Prostacyclin
PGG_2	Prostaglandin G_2
PGH_2	Prostaglandin H_2
$PGEI_2/PGI_2$	Prostaglandin I_2
PGN	Peptidoglycan
PHN	Postherpetic neuropathy
PHZ	Para-anastomotic hyper-compliant zone
PI3K	Phosphatidylinositol 3-kinase
PIP2	Phosphatidylinositol 4,5-bisphosphate
PLC	Phospholipase C
PLOD	Procollagen lysyl hydroxylase
PMCA	Plasma membrane Ca^{2+} APTases
PMN	Polymorphonuclear leukocyte
POSS	Polyhedral oligomeric silsesquioxanes
PPAR	Peroxisomal proliferation activating receptor
PPI	Proton pump inhibitor
PRV	Polycythaemia rubra vera
PS	Protein S
PSGL-1	P-selectin glycoprotein ligand-1
PT	Prothombin time
PTCA	Percutaneous coronary angioplasty
PTFE	Polytetrafluoroethylene
PTS	Post-thrombotic syndrome
PUFA	Polyunsaturated fatty acid
PVI	Primary valvular incompetence
rAAA	Ruptured AAA
Rac	Ras activated cell adhesion molecule
RANTES	Regulated upon activation, normal T cell expressed and secreted
RAS	Renin angiotensin system
RCT	Randomised controlled trial

RF	Rheumatoid factor
RFA	Radiofrequency ablation
rhAPC	Recombinant human activated protein C
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RR	Relative risk
RSD	Reflex sympathetic dystrophy
S1P	Sphingosine-1-phosphate
SAPK	Stress-activated protein kinase
SCF	Stem cell factor
SCS	Spinal cord stimulation
ScvO2	Superior vena cava venous oxygen saturation
SDF-1	Stromal-cell-derived factor-1
SERCA	Sarco/endoplasmic reticulum CaATPases
SEP	Serum elastin peptides
SES	Sirolimus-eluting stent
SEPS	Subfascial endoscopic perforator surgery
SFA	Superficial femoral artery
SFJ	Sapheno-femoral junction
SIRS	Systemic inflammatory response syndrome
SKCa	Small conductance calcium-activated potassium channels
SLE	Systemic lupus erythematosus
SMA	Smooth muscle alpha actin
SMC	Smooth muscle cell
SMP	Sympathetically maintained pain
SNARE	Soluble N-ethylmaleimide-sensitive factor activating protein receptors
SNP	Single nucleotide polymorphisms
SNRI	Serotonin/Noradrenaline reuptake inhibitors
SPJ	Sapheno-popliteal junction
SPP	Skin perfusion pressure
SR	Sarcoplasmic reticulum
SSRIs	Selective serotonin re-uptake inhibitors
SSV	Small saphenous vein

SVT	Superficial thrombophlebitis
STIM1	Stromal interacting molecule 1
TαCE	TNFα converting enzyme
TAAD	Thoracic aortic aneurysm disease
TAD	Thoracic aortic dissection
TAFI	Thrombin-activatable fibrinolysis inhibitor
Tc-99 MDP	Technetium-99 methylene diphosphonate
TCA	Tricyclic antidepressant
TCC	Total contact cast
TCR	T-cell receptor
TENS	Transcutaneous electrical nerve stimulation
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TGF	Transforming growth factor
TGF-α	Transforming growth factor-alpha
TGF-β	Transforming growth factor-beta
TGL	Triglycerides
Th	T helper
TIA	Transient ischemic attack
TIMP	Tissue inhibitors of metalloproteinase
TLR	Toll-like receptors
TNF	Tumour necrosis factor
TNF-α	Tumour necrosis factor-alpha
tPA	Tissue-type plasminogen activator
TRP	Transient receptor potential
TRPC	Transmembrane receptor potential canonical
TRPV1	Transmembrane receptor potential Vanilloid-type
TXA2	Thromboxane A2
uPA	Urokinase
UT	University of Texas
VCAM	Vascular cell adhesion molecule
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor

VEGF-R	Vascular endothelial growth factor receptor
VIP	Vasoactive intestinal peptide
VLA-1	Very late activating antigen-1
VOCC	Voltage operated calcium channels
VPT	Vibratory perception threshold
VSMC	Vascular smooth muscle cells
VTE	Venous thromboembolism
VV	Varicose veins
vWF	von Willebrand factor
XO	Xanthine oxidase

9 • Physiological Haemostasis

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INTRODUCTION

Physiological haemostasis involves complex interactions between endothelial cells, platelets and coagulation proteins, that result in a prompt platelet plug and then localised thrombus formation at the site of a break in vascular integrity. Numerous regulatory processes prevent widespread activation of coagulation, ensuring that blood remains fluid in the absence of vascular injury or other pathology. All components of the haemostatic process can be disturbed resulting in either a pro-thrombotic or bleeding tendency, and drugs that modify the haemostatic process are commonly used, particularly in patients with vascular disease. An understanding of normal haemostasis is therefore important for all clinicians that deal with this patient group.

PRIMARY HAEMOSTASIS

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

Platelets

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

Platelet adhesion

Endothelial injury results in the exposure of circulating blood to the subendothelial

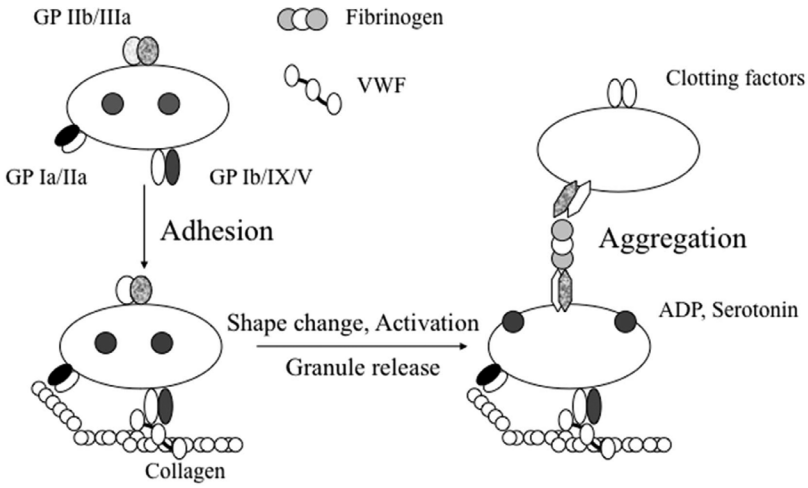

FIGURE 9.1: Mechanism of platelet aggregation

matrix that is rich in a number of adhesive proteins. von Willebrand factor (vWF) is a large adhesive glycoprotein produced by endothelial cells and megakaryocytes that is central in initial platelet adhesion.[3] The mature vWF molecule consists of disulphide-linked multimers of high molecular weight of up to 20,000,000 daltons.[4] When secreted into the plasma, these high molecular weight (HMW) vWF multimers are digested into smaller forms by the metalloprotease ADAMTS13 (a disintegrin and metalloprotease with a thrombospondin type 1 motif, member 13). These smaller soluble forms bind less readily to platelet receptors, reducing the chance of spontaneous platelet aggregation. However vWF secreted into the subendothelial space binds to other molecules such as collagen, resulting in a conformational change that exposes the binding site for platelet glycoprotein (GP) receptor Ib.[4] Subendothelial vWF is therefore 'primed' to interact with circulating platelets in the event of endothelial injury. Other important adhesive proteins include collagen type 1 and type 4, fibronectin, thrombospondin, laminin and vitronectin.

Initial platelet adhesion, particularly in high shear conditions, involves interaction between vWF and the GPIb/IX/V complex located on the platelet surface. This complex consists of four trans-membrane subunits GPIba, GPIbb, GPIX and GPV, with the N-terminal globular domain of GPIba responsible for the interaction with the A1-domain of vWF.[1] Binding of vWF to GP Ib is often reversible, and in animal models platelets can be seen to initially slide or translocate along the subendothelial surface due to cyclical attachment and then dissociation of the GP Ib/IX/V complex to vWF.[2] However, finally through further platelet receptor ligand interactions the platelet is stabilized on the subendothelial surface. The platelet glycoprotein Ia/IIa receptor (integrin $\alpha_2\beta_1$) binds collagen, an interaction that appears to be more important in low-shear conditions.[5] Glycoprotein VI, a platelet surface receptor that belongs to the immunoglobin superfamily, also directly binds collagen and further activates the GPIa/IIa receptor via intracellular signaling.[6] Other β_1 integrins also bind their respective subendothelial ligands ($\alpha_6\beta_1$ – laminin; $\alpha_5\beta_1$ – fibronectin),

and there is increasing evidence that early binding of vWF to the glycoprotein IIb/IIIa ($\alpha_{IIb}\beta_3$) receptor contributes to the initial adhesion process.[2] Finally there is evidence that formation of platelet membrane tethers, that consist of smooth cylinders of lipid membrane pulled from the platelet surface under the influence of hemodynamic drag forces, contribute to platelet adhesion in high shear conditions.[7]

Platelet activation and shape change

Following platelet adhesion, multiple pathways lead to platelet activation that results in platelet shape change, platelet granule release, and conformational change in the GP IIb/IIIa receptor that allows binding to fibrinogen and vWF, leading to platelet aggregation. Binding of vWF to the GP Ib receptor and collagen to the GP VI during the adhesion process triggers intracellular signaling via a pathway that involves activation of Src family kinases (Src), Syk and PI 3-kinase (PI3K). These events lead to the activation of phospholipase C-b (PLC), which hydrolyses membrane phospholipids to generate inositol (1,4,5) trisphosphate (IP3).[8] The binding of IP3 to its receptors (IP3R) on the dense tubular system (DTS) then results in mobilisation of intra-platelet calcium stores, which has a number of consequences including;

1. *Thromboxane A2 (TXA2) generation* – the increase in intracellular calcium stimulates the production of arachadonic acid by PLC and phospholipase A2. Arachadonic acid is converted into TxA2 via the actions of the enzymes cyclooxygenase 1 (COX-1) and Tx synthase. TxA2 is released from the platelet and binds platelet receptors TPα and TPβ. Its effects in platelets are mediated primarily through TPα. Binding of TxA2 to this G-protein coupled receptor results in further PLC activation, leading to further intracellular calcium increase further reinforcing platelet activation.[9] Local diffusion of TxA2 also contributes to the recruitment to the site of injury and activation of further platelets. Aspirin or acetyl salicylic acid exerts its antiplatelet effect by blocking TXA2 synthesis, due to the irreversible acetylation of Ser-529 in COX-1. Because platelets are anucleate, no new COX can be generated, explaining why aspirin has a persistent functional effect that lasts the lifespan of the platelet (approximately 7 days).
2. *Granule release* – intracellular calcium mobilization also results in the release from the platelet of both the dense and alpha-granules. The dense granules contain high concentrations of the small molecules adenosine diphosphate (ADP) and serotonin, which further act to reinforce local platelet activation by binding to specific platelet surface membrane receptors upon release. ADP is a central player in sustained platelet activation. The receptors for ADP, the $P2Y_1$ and $P2Y_{12}$ are seven transmembrane receptors that are coupled via heterotrimeric G-proteins to numerous intracellular effector molecules. $P2Y_1$ links to the G-protein Gq resulting in further activation of PLC and also protein kinase C activation. $P2Y_{12}$ is linked to the G-protein Gi that has an inhibitory effect on adenylate cyclase. ADP induced activation of the $P2Y_1$ receptor induces platelet shape change and rapid transient aggregation,[10] whereas activation of the $P2Y_{12}$ receptor results in sustained irreversible aggregation.[11] The thienopyridine class of antiplatelet agents, ticlopidine, clopidogrel and prasugrel exert their antiplatelet effect by blocking the $P2Y_{12}$ receptor. The active metabolites of all agents have a free thiol moiety that forms a disulfide bridge

with the extracellular cysteine residues Cys17 and Cys270.[12] Released serotonin also binds to a G-protein coupled platelet surface receptor, the 5-HT_{2A} receptor. Binding is also associated with Gq-dependent activation of PLC, resulting in amplification of platelet activation, platelet shape change, and weak reversible platelet aggregation.[13]

3. *Activation of the GP IIb/IIIa receptor* – in its resting state the GP IIb/IIIa receptor is unable to bind its ligands, namely fibrinogen and vWF. The above platelet signaling events through the activation of the small GTPase Rap1b and its interaction with a Rap1-GTP interacting adapter molecule (RIAM), lead to the binding of the proteins talin and kindlin to β3 tail of GP IIb/IIIa receptor.[14] This leads to activation of the receptor and the resulting change in conformation allows the surface portion of the receptor to bind readily to fibrinogen and vWF. The binding of talin to the receptor tail also links it to the underlying actin cytoskeleton of the platelet, enhancing adhesive strength and platelet cohesion.[15]
4. *Platelet shape change* – the normally discoid-shaped platelet with a smooth surface membrane undergoes dramatic shape change with stimulation, including extension of filopodia, and flattening or spreading on the subendothelial surface. The platelet cytoskeleton is primarily responsible for regulating the platelet's shape. Platelet activation leads to the rapid reorganization and polymerization of actin into filaments, resulting in the above conformational change.[16]

Along with ADP, the serine protease thrombin appears to play an important role in sustaining platelet activation leading to irreversible platelet aggregation. Thrombin specific receptors, the protease-activated receptors (PARs), are located on the platelet surface. Two main PARs, PAR1 a high affinity receptor and PAR4, a low affinity receptor, are involved in thrombin mediated platelet activation.[17] Thrombin activates PARs by cleaving the N-terminal of the receptor, unmasking a hidden receptor-linked ligand. This ligand then interacts with the remainder of the receptor leading to G-protein coupled signaling that results in further platelet activation.

Finally platelet activation also results in the surface expression of a number of adhesion molecules, such as the glycoprotein P-selectin which is involved in interaction with both endothelial cells and also the recruitment of inflammatory cells to the area of injury, via binding of P-selectin to P-selectin glycoprotein ligand 1 (PSGL-1) located on the surface of leucocytes.[18] Platelets also secrete chemokines such as RANTES/CCL5 and platelet factor 4 that also increases the local recruitment of inflammatory cells such as monocytes. This contributes to and can exacerbate the local inflammatory response that often presents in atherosclerotic plaque.[19]

Platelet aggregation

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

shear vascular beds and pathological arterial thrombosis.[7]

INTERACTIONS BETWEEN PRIMARY AND SECONDARY HAEMOSTASIS

While the primary and secondary haemostatic processes are often considered separately, they are intrinsically linked. As described above, the coagulation protease thrombin plays a central role in the activation of platelets. The activated platelet in turn provides the surface upon which the reaction complexes of the coagulation cascade form. In addition, as part of platelet activation the content of the negatively charged phospholipid phosphatidylserine on the outer surface of the platelet membrane increases from almost 0% up to 12%, providing a binding site for the proteins of the coagulation cascade.[20] Release of clotting factors, such as factor V, from platelet alpha granules, and the expression of other as yet still poorly defined platelet receptors for coagulation factors on the platelet surface provide additional methods in which activation of the coagulation cascade is localised to the site of platelet activation and vascular injury.[21]

SECONDARY HAEMOSTASIS

Secondary haemostasis describes the process whereby exposure of tissue factor to the bloodstream leads to a series of enzymatic reactions that result in a sufficient burst of thrombin production to convert soluble fibrinogen into a stable network. A repetitive theme in this process is the formation of a series of reaction complexes consisting of an active enzyme and a co-factor, in which the presence of the latter results in a order of magnitude increase in the efficiency of the enzyme to bind to and convert its target substrate, itself a pro-enzyme or zymogen, to its active form. Defects of secondary haemostasis, as typified by factor VIII deficiency or haemophilia A, result in muscle, joint and soft tissue bleeding, and delayed bleeding post surgical or traumatic haemostatic challenge.

The coagulation factors involved in secondary haemostasis belong to the class of proteins known as serine proteases, so called because they have a serine residue which, along with histidine and aspartic acid, forms a catalytic triad at the centre of the active site of the enzyme.[21] Most of the reactions of secondary haemostasis take place on a phospholipid membrane surface, which is normally the surface of an activated platelet. Binding of the coagulation proteins to the phospholipid membrane surface requires the presence of calcium, and agents that chelate calcium such as EDTA or citrate can therefore be utilised to prevent activation of the coagulation cascade after blood collection.

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

THE COAGULATION CASCADE

Early observations noted that clot formation in plasma would occur after the addition of exogenous biological material such as macerated brain extract, but that exposure of blood or plasma to surfaces such as glass would also precipitate clot formation without the addition of further material. This led to the concept of 'extrinsic' and 'intrinsic' pathways of coagulation, and over time the coagulation factors involved in these separate pathways were identified (Figure 9.2).[21,22] Tissue factor was identified as the 'active' factor in the added tissue extract, and was demonstrated to activate factor VII in the first part of the extrinsic pathway. The intrinsic pathway, sometimes also called the contact activation pathway, was found to involve serial activation of the coagulation factors XII, XI and IX, with factor VIII acting as a co-factor for the latter. Both extrinsic and intrinsic pathways were found to then converge on the 'common pathway' involving factor X, prothrombin (factor II), and finally the conversion of fibrinogen to fibrin. The concept of the two separate pathways was reinforced by the fact that the most widely utilised laboratory assays of coagulation evaluated the extrinsic (the prothrombin time or PT assay) and intrinsic pathway (the activated partial thromboplastin time or aPTT) separately, with both assays affected by common pathway defects.

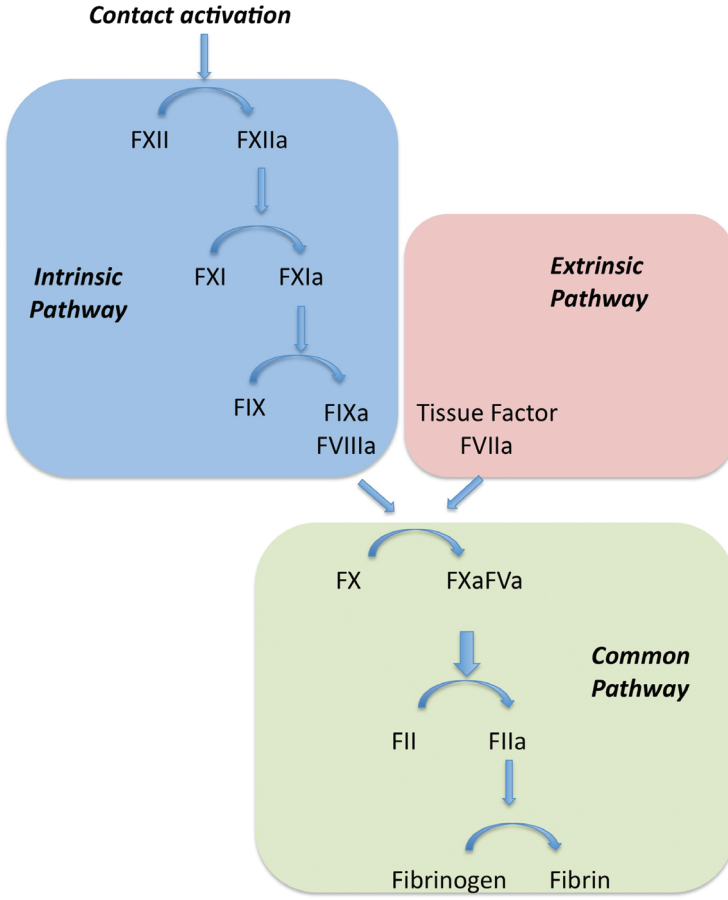

FIGURE 9.2: The extrinsic and intrinsic pathways of coagulation

It however became clear with time that the above model was unlikely to reflect physiological coagulation. The observation that inherited factor XII deficiency was not associated with a bleeding tendency raised questions regarding the physiological role of the intrinsic pathway.[23] It was also demonstrated that activated factor VII, or factor VIIa, had the ability to activate factor IX as well as factor X, and therefore that cross-talk between the pathways was likely.[24] With increasing knowledge of the role of the cell surface proteins in the coagulation process, and in particular the role of platelets, a cell-based model of haemostasis then emerged.[25] This model divides the coagulation cascade into the separate steps of initiation, amplification, and then propagation (Figure 9.3).

Initiation

Exposure of cells expressing the transmembrane protein tissue factor to circulating blood is the physiological trigger of the coagulation cascade. Tissue factor (TF) is a transmembrane protein that is constitutively expressed on the surface of most non-vascular cells, including those located in the subendothelium. There is also some evidence that tissue factor expression can be induced in the setting of inflammation on the surface of monocytes and that microparticles derived from monocytes may also express TF in pathological states.[26] Upon exposure to circulating blood TF binds to factor VII, converting it to its active form factor VIIa. The resulting enzymatic structure is known as the extrinsic tenase complex, with TF then acting as a co-factor for VIIa and greatly potentiating conversion of factor X to factor Xa, and, to a lesser degree, factor IX to factor IXa. The activated factor Xa formed then binds to the surface of the tissue factor-expressing cell, and converts a small amount of prothrombin (factor II) to thrombin, while the small amount of factor IXa produced diffuses away with the potential to bind locally to the surface of activated platelets.[27]

Amplification

The small amount of thrombin formed during the initiation stage, while insufficient

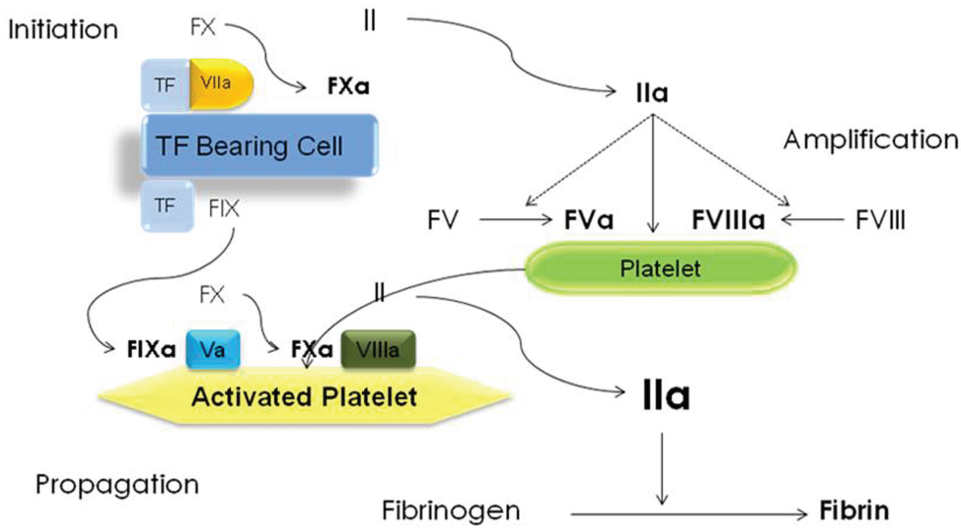

FIGURE 9.3: Cell based model of haemostasis

to convert adequate amounts of fibrinogen to fibrin, is none-the-less enough to be responsible for the subsequent amplification of the coagulation cascade. The thrombin produced results in 1) further local activation of platelets resulting in the phospholipid surface on which the reactions of the coagulation cascade can proceed; 2) activation of the co-factors factor V and factor VIII that then localize on the nearby surface of activated platelets; and 3) activation of factor XI that also binds locally to the platelet surface.[28]

Propagation

Following the activation of the co-factors and their localization on the platelet surface, the stage is set for the formation of highly efficient enzymatic complexes that are responsible for the burst of thrombin generation that leads to clot formation. Factor IXa formed during the initiation step, binds to factor VIIIa on the platelet surface to form the intrinsic tenase complex. This then efficiently converts factor X to factor Xa, with the latter then binding to its co-factor, factor Va, to form the prothrombinase complex responsible for the effective conversion of prothrombin to thrombin. Factor XIa produced during amplification activates further factor IX, further reinforcing or enhancing the whole process from above.[25]

The burst of thrombin generated during propagation then cleaves the fibrinopeptides a and b from soluble fibrinogen to form insoluble fibrin monomers. The transglutaminase Factor XIII, itself activated by thrombin, then forms bonds between separate fibrin monomers to form a firm network of cross-linked fibrin that is a requirement for stable thrombus formation.[29]

Natural inhibitors of coagulation

Normal coagulation is kept in check by several regulatory processes that cause thrombin production to plateau and then diminish, preventing appropriate localized activation of coagulation from becoming an inappropriate widespread activation of the clotting cascade. The initiation phase of coagulation is regulated by tissue factor pathway inhibitor (TFPI), a protein produced by endothelial cells.[30] After a sufficient local concentration of FXa is generated in the initiation step of coagulation, TFPI is able to form an inhibitory quaternary complex with FXa, FVIIa, and tissue factor, preventing continued activation of the cascade from above.

Central to regulation of the propagation phase of the coagulation cascade is the protein C anticoagulant pathway that involves protein C and protein S, both vitamin K dependent plasma glycoproteins synthesized in the liver.[31,32] Thrombin itself initiates this inhibitory pathway after binding to thrombomodulin, a transmembrane protein located on the intact endothelial cell surface in all vascular beds particularly in the microcirculation. Binding of thrombin to thrombomodulin results in a change in substrate specificity that favours cleavage of the vitamin K dependent protein C to its activated form activated protein C (APC).[33] Binding of thrombin to thrombomodulin therefore results in its net enzymatic effect being switched from pro-coagulant to anticoagulant. Another endothelial transmembrane protein, the endothelial protein C receptor (EPCR) binds protein C, helping to localize the protein at the endothelial surface potentiating activation by thrombomodulin bound thrombin. Once activated APC diffuses away from EPCR, and binds to the extrinsic tenase and prothrombinase complexes where it acts to inactivate factor VIIIa

and factor Va respectively. Protein S acts as a co-factor for protein C in these reactions, as well as having some direct anticoagulant activity.[34] In plasma, PS circulates both free (40%) and bound to the C4b-binding protein (60%). It is the free form of PS that has cofactor activity.[32]

Finally antithrombin (AT) is a single chain plasma glycoprotein that belongs to the serine protease inhibitor superfamily (serpins). It plays a central role in the inactivation of circulating activated clotting factors, forming a 1:1 complex that is cleared by the liver. It is the main physiological inhibitor of thrombin and also binds to factors Xa, IXa, XIa, and XIIa.[35] Thrombin inhibition by AT is potentiated more than 1000-fold by heparin, due to conformational change of the AT molecule upon heparin binding, and it is this mechanism that results in heparin's activity as an anticoagulant agent.[36]

Inherited deficiency states of the main inhibitory proteins of coagulation, namely protein C, protein S and antithrombin, have all been described, and result in a prothrombotic tendency. Such deficiency states are relatively rare accounting, when combined, for less than 5% of individuals with venous thrombosis in a Caucasian population.

Fibrinolysis

The fibrinolytic system is responsible for the dissolution of thrombus composed of cross-linked fibrin, and plays a major role in helping maintain a patent vascular system.[37] It is composed of a number of enzymes, most of which are serine proteases, that act in concert to convert insoluble fibrin to soluble fibrin degradation products (FDPs). The central protein of the fibrinolytic system is plasminogen, a single-chain glycoprotein consisting of 791 amino acids, which is converted to its active form plasmin by the cleavage of a single Arg561–Val562 peptide bond. Tissue-type plasminogen activator (tPA) is the physiological activator primarily involved in the dissolution of fibrin from the circulation. Activation of plasminogen to plasmin is potentiated in the presence of fibrin due to the fact that both plasminogen and tPA bind to lysine residues on the surface of fibrin, and are as a result brought into close proximity to each other. Both tPA and another plasminogen activator, urokinase-type plassminogen activator, play a role in the activation of plasminogen that is bound to the endothelial cell surface. Once activated, plasmin cleaves fibrin into soluble fibrin degradation products, of which D-dimer is one. D-dimer consists of two cross-linked fibrin D-domains, and is not normally present in the absence of recent plasmin activity. It is therefore used as a laboratory marker of active thrombosis, and is a sensitive test that can be used to rule out recent venous thromboembolism.

Like the coagulation cascade, the fibrinolytic system also has a number of inhibitory proteins that in normal circumstances prevent widespread activation of fibrinolysis. Plasminogen activator inhibitor-1 (PAI-1) is a 52-kd, single-chain glycoprotein that belongs to the serpin family, that is the main inhibitor of both tPA and uPA, doing so by forming a 1:1 complex that is cleared by the liver.[39] Circulating plasmin is quickly mopped up by α_2-plasmin that is present in the circulation at a high concentration. The most recently described inhibitor of fibrinolysis is thrombin-activatable fibrinolysis inhibitor (TAFI), a carboxypeptidase.[40] TAFI is activated by thrombin, a process that is markedly accelerated if thrombin is bound to thrombomodulin. The antifibrinolytic activity of TAFI is due the fact that it cleaves C-terminal lysine and arginine residues from fibrin. This significantly reduces the binding of plasminogen to fibrin, therefore decreasing

the activation of plasminogen by tPA on the surface of the fibrin clot.

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

CONCLUSIONS

Primary and secondary haemostasis both involve carefully balanced systems that if disturbed can lead to issues with either bleeding or pathological thrombosis. An improved understanding of the molecular processes involved has lead to the development of more targeted therapeutic options, such as the direct thrombin inhibitors and direct factor Xa inhibitors, with the aim of increasing the benefit and reducing the risks associated with anticoagulation. Continued advances in our understanding of the relationship between the structure and function of the proteins and receptors involved in haemostasis, along with improved technology, is likely to lead to further therapeutic advances in coming decades.

REFERENCES

1. 'Löwenberg EC, Meijers JCM, Levi M. Platelet-vessel wall interaction in health and disease. *Neth J Med* 2010 Jun; **68**(6): 242–51.
2. Jackson S. The growing complexity of platelet aggregation. *Blood* 2007; **109**: 5087.
3. Ruggeri ZM. Structure and function of von Willebrand factor. *Thromb Haemost* 1999; **82**(2): 576–584.
4. Ware JA, Heistad DD. Seminars in medicine of the Beth Israel Hospital, Boston. Platelet-endothelium interactions. *N Engl J Med* 1993; **328**(9): 628–35.
5. Jung SM, Moroi M. Activation of the platelet collagen receptor integrin alpha(2)beta(1): its mechanism and participation in the physiological functions of platelets. *Trends Cardiovasc Med* 2000; **10**(7): 285–92.
6. Clemetson KJ, Clemetson JM. Platelet collagen receptors. *Thromb Haemost* 2001; **86**(1): 189–97.
7. Jackson SP, Nesbitt WS, Westein E. Dynamics of platelet thrombus formation. *J Throm Haemost* 2009; **7** (Suppl. 1): 17–20.
8. Varga-Szabo D, Pleines I, Nieswandt B. Cell adhesion mechanisms in platelets. *Arterioscler Thromb Vasc Biol* 2008; **28**(3): 403–412.
9. Offermanns S. Activation of platelet function through G protein-coupled receptors. *Circ Res* 2006; **99**(12): 1293–1304.
10. Fabre JE, Nguyen M, Latour A, et al. Decreased platelet aggregation, increased bleeding time and resistance to thromboembolism in P2Y1-deficient mice. *Nat Med* 1999; **5**(10): 1199–1202.
11. Dorsam RT, Kunapuli SP. Central role of the P2Y12 receptor in platelet activation. *J Clin Invest* 2004; **113**(3): 340–345.
12. De Meyer SF, Vanhoorelbeke K, Broos K, Salles II, Deckmyn H. Antiplatelet drugs. *Br J Haem* 2008; **142**: 515–528.
13. Li N, Wallen NH, Ladjevardi M, Hjemdahl P. Effects of serotonin on platelet activation in whole blood. *Blood Coag Fibrin* 1997; **8**: 517–523.
14. Watanabe N, Bodin L, Pandey M, Krause M, Coughlin S, Bous- siotis VA, Ginsberg MH, Shattil SJ. Mechanisms and consequences of agonist-induced

talin recruitment to platelet integrin $\alpha_{IIb}ß_3$. *J Cell Biol* 2008; **181**: 1211–22.
15. Shattil SJ. The β3 integrin cytoplasmic tail: protein scaffold and control freak. *J Thromb Haemost* 2009; **7** (Suppl. 1): 210–3.
16. Fox JEB. Cytoskeletal proteins and platelet signaling. *Thromb Haemost* 2001; **86**: 198–213.
17. Coughlin SR. Protease-activated receptors in hemostasis, thrombosis and vascular biology. *J Thromb Haemost* 2005; **3**: 1800–1814.
18. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. *J Clin Invest* 2005; **115**(12): 3378–3384.
19. May AE, Seizer P, Gawaz M. Platelets: inflammatory firebugs of vascular walls. *Arterioscler Thromb Vasc Biol* 2008; **28**(3): s5–s10.
20. Beavers EM, Comfurius P, Zwaal RFA. Changes in membrane phospholipid distribution during platelet activation. *Biochem Biophys Acta* 1983; **736**: 57–66.
21. Macfarlane RG. An enzyme cascade in the blood clotting mechanism, and its function as a biological amplifier. *Nature* 1964; **202**: 498–9.
22. Davie EW, Ratnoff OD. Waterfall sequence for intrinsic blood clotting. *Science* 1964; **145**: 1310–2.
23. Roberts HR, Monroe DM, Oliver JA, Chang JY, Hoffman M. Newer concepts of blood coagulation. *Haemophilia* 1998; **4**: 331–4.
24. Østerud B, Rapaport SI. Activation of factor IX by the reaction product of tissue factor and factor VII: additional pathway for initiating blood coagulation. *Proc Natl Acad Sci* USA 1977; **74**: 5260–4.
25. Hoffman M, Monroe DM. A Cell-based Model of Hemostasis. *Thromb Haemost* 2001; **85**: 958–65.
26. Key NS, Mackman N. Tissue Factor and Its Measurement in Whole Blood, Plasma, and Microparticles. *Semin Thromb Haem* 2010; **36**(8): 865–875.
27. Hoffman M, Monroe DM, Oliver JA, et al. Factors IXa and Xa play distinct roles in tissue factor-dependent initiation of coagulation. *Blood* 1995; **86**: 1794–801.
28. Monroe DM, Roberts HR, Hoffman M. Platelet procoagulant complex assembly in a tissue factor-initiated system. *Br J Haematol* 1994; **88**: 364–71.
29. Board PG. Losowsky MS. Factor XII: Inherited and acquired deficiency. *Blood Rev* 1993; **7**: 229–242.
30. Baugh RJ, Broze GJ Jr, Krishnaswamy S. Regulation of extrinsic pathway factor Xa formation by tissue factor pathway inhibitor. *J Biol Chem* 1998; **273**: 4378–4386.
31. Esmon CT. The protein C pathway. *Chest* 2003; **124**: 26S–32S.
32. Lane DA, Mannucci PM, Bauer KA, et al. Inherited thrombophilia: Part 1. *Thromb Haemost* 1996; **76**: 651–62.
33. Esmon CT. The roles of protein C and thrombomodulin in the regulation of blood coagulation. *J Biol Chem* 1989; **264**: 4743–4746.
34. Heeb MJ, Mesters RM, Tans G, et al. Binding of protein S to factor Va associated with inhibition of prothrombinase that is independent of activated protein *C. J Biol Chem* 1993; **268**: 2872–7.
35. Bayston TA, Lane DA. Antithrombin: molecular basis of deficiency. *Thromb Haemost* 1997; **78**: 339–43.
36. Hirsh J, Raschke R. Heparin and low-molecular-weight heparin: the Seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. *Chest* 2004; **126**: 188S–203S.

37. Rijken DC, Lijnen HR. New insights into the molecular mechanisms of the fibrinolytic system. *J Thromb Haemost* 2009; **7**: 4–13.
38. Castellino FJ, Ploplis VA. Structure and function of the plasminogen/plasmin system. *Thromb Haemost* 2005; **93**: 647–54.
39. Pannekoek H, Veerman H, Lambers H, Diergaarde P, Verweij CL, van Zonneveld AJ, van Mourik JA. Endothelial plasminogen activator inhibitor (PAI): a new member of the Serpin gene family. *EMBO J* 1986; **5**: 2539–44.
40. Nesheim M, Bajzar L. The discovery of TAFI. *J Thromb Haemost* 2005; **3**: 2139–46.

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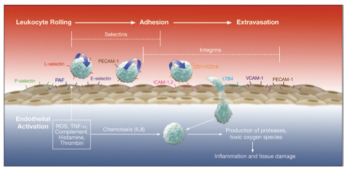

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