

MECHANISMS OF VASCULAR DISEASE:

A REFERENCE BOOK FOR VASCULAR SPECIALISTS



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Mechanisms of Vascular Disease

Mechanisms of Vascular Disease:

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 | Adrenocorticotrophic 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 |
| β 2-GPI | β 2-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 | Calcitonin 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-1 α | 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 <i>Staphylococcus aureus</i> |
| MRSE | Methicillin resistant <i>Staphylococcus epidermidis</i> |
| 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 |
| T α CE | TNF α 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

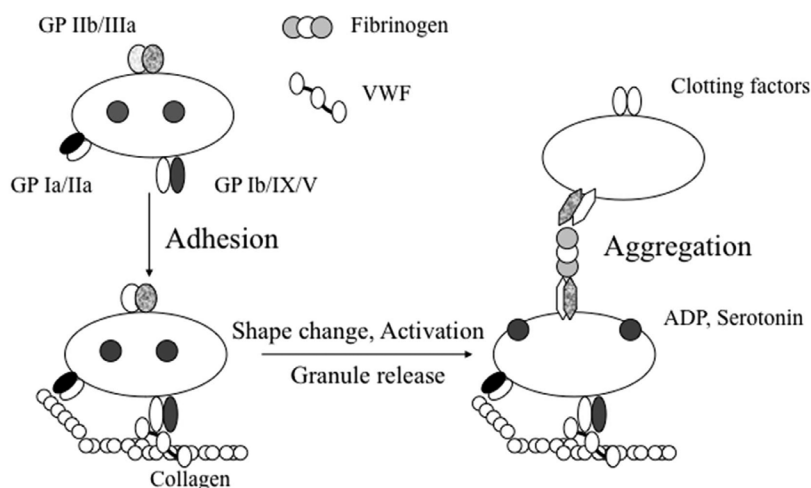


FIGURE 9.1: Mechanism of platelet aggregation

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 immunoglobulin 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- β (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 TP α and TP β . Its effects in platelets are mediated primarily through TP α . 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 P2Y₁ and P2Y₁₂ are seven transmembrane receptors that are coupled via heterotrimeric G-proteins to numerous intracellular effector molecules. P2Y₁ links to the G-protein Gq resulting in further activation of PLC and also protein kinase C activation. P2Y₁₂ is linked to the G-protein Gi that has an inhibitory effect on adenylate cyclase. ADP induced activation of the P2Y₁ receptor induces platelet shape change and rapid transient aggregation,¹⁰ whereas activation of the P2Y₁₂ receptor results in sustained irreversible aggregation.¹¹ The thienopyridine class of antiplatelet agents, ticlopidine, clopidogrel and prasugrel exert their antiplatelet effect by blocking the P2Y₁₂ 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 $\beta 3$ 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.

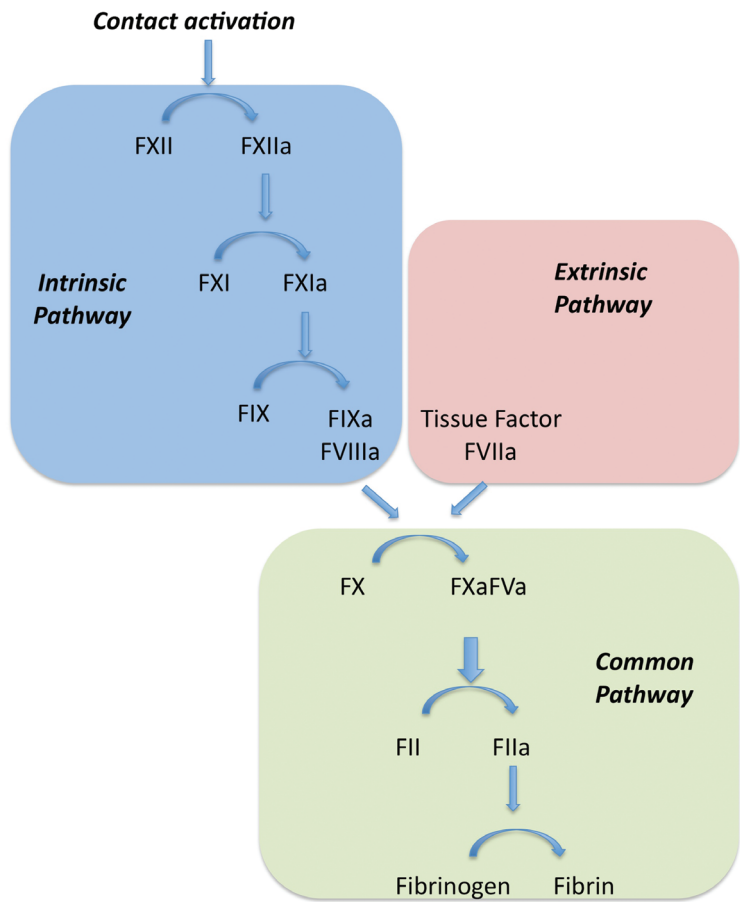


FIGURE 9.2: The extrinsic and intrinsic pathways of coagulation

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

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 pro-thrombotic tendency. Such deficiency states are relatively rare accounting, when combined, for less than 5% of individuals with venous thrombosis in a Caucasian population.

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

Like the coagulation cascade, the fibrinolytic system also has a number of inhibitory proteins that in normal circumstances prevent widespread activation of fibrinolysis. Plasminogen activator inhibitor-1 (PAI-1) is a 52-kd, single-chain glycoprotein that belongs to the serpin family, 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

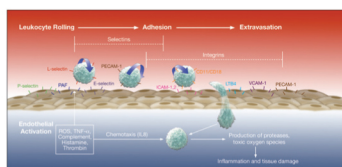
Primary and secondary haemostasis both involve carefully balanced systems that if disturbed can lead to issues with either bleeding or pathological thrombosis. An improved understanding of the molecular processes involved has led to the development of more targeted therapeutic options, such as the direct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

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