Thrombosis

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Introduction

The human hemostatic system has evolved to maintain blood flow under normal physiologic conditions while remaining primed to rapidly respond to vascular injury with a burst of activity that ceases blood leakage by the formation of a clot. In addition, regulatory mechanisms are designed to prevent the continuous propagation of the clot and to dissolve existing clots.

This delicate balance of clotting and anti-clotting factors is dependent on the interaction of:

1. Endothelial cells
2. Platelets (and other cellular elements)
3. Plasma proteins
   a. Procoagulant (clot forming)
   b. Anticoagulant (clot inhibiting)
   c. Fibrinolytic (clot-lysing)

Endothelial Thromboregulation

Endothelial cells play a very important role in maintaining normal blood flow. Their anti-thrombotic properties include anticoagulant, anti-platelet, and fibrinolytic properties. The endothelial lining of the blood vessel establishes a physical barrier between the components of blood and the highly reactive elements that are present in the subendothelium, which most importantly contains von Willebrand factor (vWF), collagen and tissue factor (TF) bearing cells. Endothelial cells are also highly negatively charged and repel the negatively charged platelets and prevent them from adhering to the surface.

Natural anti-platelet factors are produced by endothelial cells such as nitric oxide (NO), prostacyclins (PGI₂), and nucleotidases (CD39 and CD73). NO is a well-known vasodilator and works by relaxing the vascular smooth muscle is also a well-known inhibitor of platelets activation. PGI₂ mediates the inhibition of platelet aggregation and disaggregation of existing platelet aggregates. The nucleotidases CD39 and CD73 are endothelial membrane bound proteins that block platelet aggregation by degrading ADP released from platelets.

In addition to the anti-platelet properties, the endothelium also has significant anticoagulant properties:
1. Endothelial-derived heparin sulfate, a linear polysaccharide, accelerates antithrombin (AT) activity to decrease thrombin (IIa), factor Xa, and other activated factor functional activity.

2. The presence of thrombomodulin, an endothelial transmembrane glycoprotein, is a cofactor that increases the activity of Protein C and its cofactor Protein S resulting in the break down of factor Va and factor VIIIa.

3. Plasminogen is converted to plasmin by tissue plasminogen activator (tPA) produced by endothelial cells to initiate clot lysis.

Alteration of normal thromboregulation at the site of vascular injury leads to the loss of the usually anti-clotting mechanisms. Vasoconstriction leads to stasis and diversion of blood away from the site of injury. Altered endothelial synthesis of NO, PGI₂ and ADPases result in the loss of the potent antiplatelet effect. Activation of the endothelial cell will also create a procoagulant surface and activate secondary hemostasis. The loss of the endothelial layer integrity is critical in exposing blood elements to the subendothelium, which most importantly contains collagen, von Willebrand factor, and tissue factor, a potent stimulator of secondary hemostasis that is expressed on endothelial cells, smooth muscle, fibroblasts and macrophage. This exposure to the subendothelial elements initiates primary hemostasis, which is the formation of the initial platelet plug.

**Primary Hemostasis**

Following damage to the endothelium (e.g. blood vessel trauma) platelets are exposed to the subendothelial compartment and form the primary hemostatic (platelet) plug.

The most important steps in the formation of the platelet plug include:

1. **Adhesion**
2. **Activation and Recruitment**
3. **Aggregation**

**Adhesion**

The exposure of the blood to the subendothelium is critical for the initial of adhesion of platelets since they normally are repelled and inactivated by intact, uninjured endothelial cells. Platelets passing the site of injury will adhere under high shear stress conditions to von Willebrand factor via the platelet surface glycoprotein GP Ib-IX-V. Platelet binding to collagen under conditions of low shear stress is mediated by the platelets receptors GP VI and CD36, but is thought to be of lesser importance in vivo. In the inherited disorder Bernard-Soulier Disease, the congenital absence of the GPIb-IX-V complex results in a severe bleeding disorder due to loss of platelet adherence to the site of injury.
**Activation, Release Reaction, Recruitment**

The binding of the platelets to subendothelial collagen and von Willebrand factor initiates a sequence of intracellular signals that results in the activation of the platelet. Platelets in the quiescent state are discoid in shape, but upon activation the platelets spread across the site of adhesion. This increases the platelet membrane surface area that is exposed at the site of injury. Although the shape change is evident on electron microscopy, a morphologic event occurs that can not be seen by EM. The platelet membrane in its quiescent state is asymmetric with a much greater number of phosphatidyl serine molecules present in the inner leaf of the membrane than the outer leaf. However, after activation, enzymes, such as flippase and floppase, change the orientation of the membrane so that the negatively charged phospholipid surface is exposed on the outer leaflet. Calcium is also released from the platelet; the combination of the phosphatidylserine and calcium provide the necessary conditions for coagulation reactions.

In addition, platelets release soluble platelet agonists that are stored in the platelet dense granules and alpha granules. ADP is one of the more potent agonists that are released. Thromboxane is also synthesized by the conversion of arachidonic acid by the cyclo-oxygenase enzymes (COX). Other soluble agents, including soluble agonists, that are released include (and a lot of others that can’t be covered here):

1. **Dense Granules**
   a. Calcium, Magnesium, Pyrophosphate
   b. ATP, GTP, ADP, GDP
   c. Serotonin

2. **Alpha Granules**
   a. Von Willebrand factor
   b. Fibrinogen
   c. Chemokines
   d. Factor V and factor VIII
   e. Plasminogen, Plasminogen activator inhibitor

Defects in many of the activation pathways, inherited or acquired, have been described. (e.g. Hermansky-Pudlak Syndrome, Inherited disorders of the receptors for platelet agonists). Anti-platelet medications have also been designed to inhibit these release and activation reactions. Commonly used in cerebrovascular and cardiovascular disease, clopidogrel (Plavix) and ticlopidine (Ticlid) block the platelet ADP receptor and aspirin inhibits COX and therefore thromboxane production.

**Aggregation**

The activation of platelets leads to the increased production and activity of the binding of platelet glycoprotein receptor GPIIb/IIIa to the plasma protein
fibrinogen. The bridging of fibrinogen to other platelets leads to aggregation of platelets and the formation of the platelet plug. Clansman’s Thrombasthenia is an inherited disorder of the GPIIb/IIIa receptor and results in a severe bleeding disorder. In addition, GPIIb/IIIa antagonists such as eptifibatide and abciximab have been designed to block this receptor and are commonly used in interventional cardiac procedures to prevent coronary artery re-occlusion.

**Secondary Hemostasis**

The initial platelet plug is stabilized into the definitive hemostatic clot by the initiation of the coagulation cascade. The final endpoint is the conversion of fibrinogen to fibrin by a thrombin (factor IIa) burst that results from a cascade of enzymatic activity on the surface of cellular elements such as platelets and endothelial cells. Zymogens are cleaved into active enzymes, which in turn cleave the next enzyme – positive feedback occurs so that the reactions accelerate. Several of the important factors in this cascade are vitamin K dependent coagulation factors (factor II, VII, IX, X and Protein C and Protein S). During synthesis in the liver, these enzymes require carboxylation for functional activity, which in turn requires vitamin K to recycle the enzymes. Vitamin K deficiency can lead to functional deficiencies of factor II, VII, IX, and X, as well as the anticoagulant factors protein C and protein S. Vitamin K is the target of the anticoagulant medication warfarin (Coumadin).

The coagulation cascade is originally based on in vitro testing systems (e.g. prothrombin time) and has been traditionally taught as the intrinsic, extrinsic, and common pathways. However, this model has been known since its creation to be untenable in explaining our observations of in vivo hemostasis and thrombosis. For example, why don’t patients with high molecular weight kininogen, Prekallikrein, or factor XII deficiencies have bleeding disorders (in fact, they may even be associated with thrombosis). Newer models have been developed to incorporate our understanding that that TF:VIIa is a potent activator of factors IX and X and is the most important pathway for coagulation initiation. More complete models of coagulation include the cellular compartments to incorporate our knowledge that TF:VIIa activity occurs cells different than from the reactions that take place on the platelet surface.

The classical teaching model will be discussed here for a brief introduction to hemostasis and thrombosis. The intrinsic pathway is the model that is used to explain why blood placed into a glass beaker clots. The clotting depends on the activation of clotting by factors “intrinsic” to the blood. In vitro, surface activation by glass (or other activating surface) results in prekallikrein and high molecular weight kininogen converting factor XII to XIIa, which cleaves factor XI to Xa, which then cleaves factor IX to IXa. Factor IXa complexes with factor VIIIa, calcium and phospholipids to form the “tenase complex” (IXa:VIIIa:Ca++:PL), which cleaves X to Xa. The cleavage of X to Xa begins the common pathway. The “prothrombinase complex” forms when factor Xa, factor Va, calcium and phospholipids (Xa:Va:Ca++:PL) combine. This complex plays a critical role in cleaving prothrombin (II) to thrombin (IIa). The formation of the “thrombin burst”
is most important final pathway of hemostasis as it converts fibrinogen to fibrin to form a stable clot. Thrombin is also a very potent activator of platelets, provides a strong positive feedback mechanism by cleaving many of the preceding zymogens, and also activates many of the regulatory factors to limit the growth of the clot.

The extrinsic pathway (tissue factor pathway) explains clotting initiated by a cut. Tissue factor, extrinsic to blood, combines with activated VIIa to form TF:VIIa, which cleaves X to Xa in the common pathway. However, in vivo, tissue factor most likely activates both factor IX and X

**Regulation (Anticoagulant and Fibrinolytic Factors)**

If platelet activation and coagulation were allowed to continue unchecked the clot would propagate and completely occlude the blood vessels. Therefore, a series of regulatory proteins limit the continued growth of the clot. For example, NO production by platelets after activation limits aggregation. Important enzymes in the coagulation cascade include antithrombin and protein C and protein S. AT limits coagulation by cleaving thrombin and Xa and with less potent activity against other activated factors. AT by itself is a very slow enzyme, but heparin-like molecules significantly accelerate its activity. Activated protein C requires the protein S to break down factors VIIIa and Va, thus limiting coagulation. The anticoagulant tissue factor pathway inhibitor and thrombomodulin are present on endothelial cells.

In addition, clots do not occupy the site of injury forever. While tissue repair takes place, the activation of fibrinolysis leads to the lysis of fibrin into fibrin degradation products (FDPs) such as D-dimer. Endothelial cells produce plasminogen activators such as tissue plasminogen activator (t-PA), which cleaves plasminogen to plasmin. Plasmin then breaks down fibrin.

**Thrombosis**

Thrombi can develop anywhere in the circulatory system and complications can result from the local obstruction of the vessel, dislodgement of thrombi with downstream embolization, occlusion, and ischemic injury, or from consumption of coagulation and anticoagulation factors (e.g. disseminated intravascular coagulation). Arterial thrombi more commonly develop at sites of pre-existing vessel injury such as atherosclerotic disease. The acute onset of these thrombi in the coronary arteries is one of important mechanisms that cause acute coronary syndrome such as unstable angina and acute myocardial infarction. Arterial thrombi are also seen in disorders such as heparin induced thrombocytopenia, the antiphospholipid antibody syndrome, and hyperhomocysteinemia. These clots can continue to propagate and form larger and larger thrombi, eventually obstructing the vessel, embolize and create distal ischemia, resolve, or organize with the migration of fibroblasts and other cells into the thrombus.
Rudolph Virchow noted in the 1800’s that venous thromboembolism required damaged endothelium, stasis, and a hypercoagulable state. This model is still the basis for our understanding of the pathophysiology of thrombus formation. When the delicate balance of antithrombic and prothrombic factors is altered by an inherited or acquired prothrombotic disorder, this balance tips normal hemostasis into excessive thrombosis.

**Virchow’s Triad**

1. **Endothelial Damage**
2. **Vascular Stasis**
3. **Hypercoagulable State**

Endothelial damage is often only thought of as mechanical injury that denudes the endothelium. However, chemical influences, for example endotoxin, inflammatory states, immune complexes, and smoking, can also lead to changes in endothelial cells, leakage of blood components into the subendothelium, and loss of the other protective thromboregulatory mechanisms.

Stasis may be the result of a change in the vascular structure and reduced or turbulent blood flow. Examples include atherosclerosis and aortic aneurysms that can cause turbulence or local stasis and immobilization. Acute myocardial infarction can cause local stasis if ventricular wall aneurysms develop.

Hypercoagulable states, which can be inherited or acquired, predispose patients to thrombosis. However, in most instances these states generally do not cause thrombosis alone.

**“Two Hit” Hypothesis**

Currently, the initiation of thrombosis is thought to require more than one risk factor to manifest thrombosis in most patients.

**Congenital Risk Factor + Acquired Risk Factor → Thrombosis**

e.g. Factor V Leiden Mutation + Oral Contraceptive → Thrombosis

**Inherited Thrombophilias**

- Factor V Leiden (Activated Protein C Resistance) – most common inherited thrombophilia
- Prothrombin G20210A mutation
- Congenital Hyperhomocysteinemia
- Protein C deficiency
- Protein S deficiency
- Antithrombin deficiency
• Plasminogen deficiency

Acquired Hypercoagulable State
• Immobilization (e.g. prolonged flight)
• Surgery
• Trauma
• Malignancy
• Pregnancy
• Oral Contraceptives
• Estrogen Replacement Therapy
• Antiphospholipid Antibodies
• Obesity
• Smoking
• Polycythemia Vera (hyperviscosity)
• Cardiac and Vascular Disease (atrial fibrillation, 

Arterial Thrombosis
Arterial thrombi are more likely to form with certain types of pathology. For example, the hypercoagulable states resulting from antiphospholipid antibodies, hyperhomocysteinemia, and heparin induced thrombocytopenia can cause arterial thrombosis in addition to arterial thrombosis. Arterial damage or vessel damage can also lead to thrombosis. Ruptured plaques form a nidus for thrombus formation and the resulting acute coronary syndrome. The pathophysiology of coronary artery occlusion is the rupture of unstable plaques, which activate platelet plug formation and secondary Hemostasis resulting in occlusion of the coronary vessel. Localized stasis can be seen after myocardial infarction with the formation of a mural thrombus and in atrial fibrillation where clots can develop in the left atrial appendage. Arterial thrombosis can also be a result of venous thromboembolism. Paradoxical emboli occur when a venous thrombus dislodges (e.g. deep vein thrombosis) and rather than pass into the pulmonary circulation, the thrombus will cross a defect in the heart, most commonly a patent foramen ovale, and cause arterial occlusion such as an embolic stroke.

Venous Thrombosis and Venous Thromboembolism
Venous thrombosis can develop in the deep veins such as the iliac and femoral veins. This is especially a problem in hospitalized patients where long-term immobilization after surgery can lead to venous stasis. The first hit may be an underlying Factor V Leiden mutation and the second hit may be venous stasis during an intercontinental flight. This “second hit” can result in the formation of a deep venous thrombosis. Deep venous thrombosis can lead to phlegmasia or edema of the lower extremity and swelling and inflammation. It is much more life-threatening if the thrombus dislodges and to pulmonary vasculature (pulmonary thromboembolism). Such clots will become trapped in the pulmonary artery and prevent blood flow to the lung tissue supplied by the pulmonary artery
leading to respiratory and circulatory compromise. A large venous thromboembolism that becomes lodged in the main pulmonary artery bifurcation and is termed a saddle embolus (i.e. it saddles the bifurcation of the artery).

**Morphology**

Thrombi can develop anywhere in the circulatory system – venous thrombus, arterial thrombus, microcirculation, mural (e.g. post-myocardial infarction). Thrombi consist of fibrin and cellular blood elements with proportions of these elements varying depending on the site of the injury. For example, in locations with stasis such as venous thrombosis, a considerable amount of entrapped red blood cells and fewer platelets are morphologically seen interspersed with strands of fibrin. Layers of platelets, fibrin, and other cellular elements can be seen (Lines of Zahn).

**Anticoagulation and Antiplatelet Therapy**

Two commonly used anticoagulants include heparin and warfarin (Coumadin). Heparin, in its unfractionated form, is a long polysaccharide and is most commonly administered intravenously, although low molecular weight heparin is administered subcutaneously. These long polysaccharide chains enhance the activity of antithrombin resulting in the inhibition of the coagulation cascade, most potently against thrombin and factor Xa. Since patients on intravenous heparin become outpatients, the oral anticoagulant, warfarin, is most commonly used in the outpatient setting. Warfarin is a vitamin K antagonist that prevents the carboxylation of coagulation factors II, VII, IX, and X, as well as the anticoagulation factors Protein C and Protein S, which leads to non–functioning proteases. Other anticoagulants available include direct thrombin inhibitors such as argatroban, hirudin, and ximelegatran.

Antiplatelet agents are commonly used for primary and secondary prophylaxis of arterial thrombi that cause acute coronary syndromes, acute neurovascular syndromes, and peripheral artery disease. Aspirin (ASA), which irreversibly inhibits the production of thromboxane by inhibiting cyclooxygenase, impairs this potent platelet agonist and prevents the recruitment of additional platelets. ADP receptors (e.g. P2Y12) can be blocked by clopidogrel (Plavix) and ticlopidine (Ticlid), thus blocking activation by released ADP by activated platelets. Aggregation can be inhibited by the GPIIb/IIIa antagonists Reopro (abciximab) and Integrelin (eptifabatide), which prevent the formation of platelet–fibrinogen-platelet bridging.