Abstract—Hemostasis is a normal process preventing the sequelae of uncontrolled hemorrhage. In certain settings, these same processes cause adverse clinical events due to thrombotic occlusion of a vessel. The majority of unstable coronary syndromes result from disruption of an atherosclerotic plaque, leading to the exposure of subintimal contents, which triggers coagulation and the formation of a platelet-rich thrombus. The central role of platelet activation in the events that lead to vessel occlusion is well known. However, this process is complex and influenced by a myriad of cellular and plasma-derived mediators that regulate the balance between occlusive and nonocclusive thrombosis. (Circulation. 2005;112:2725-2734.)

Key Words: coagulation ■ platelets ■ thrombosis

Thrombosis of either the venous or arterial circulation is a major cause of morbidity and mortality. Under normal circumstances, the hemostatic process is a delicate balance of prothrombotic and antithrombotic factors in the vasculature. Normal hemostasis prevents uncontrolled hemorrhage; however, these same pathways regulating hemostasis lead to pathological thrombosis and vessel occlusion. Advances in understanding the mechanisms of thrombosis, as well as the development of new techniques for studying its regulation, have led to a clearer understanding of thrombotic disease.

Thrombus formation within a vessel is the precipitating event in multiple vascular disease processes, including myocardial infarction, thrombotic cerebrovascular events, and venous thrombosis; however, the pathophysiological processes regulating these diseases are distinct. In venous thrombosis, primary hypercoagulable states reflecting defects of the proteins of coagulation and fibrinolysis or secondary hypercoagulable states involving abnormalities of blood vessels and blood flow lead to thrombosis (see the recent review by Andreotti and Becker1). Distinct from venous thrombosis, arterial thrombosis is highly dependent on the vessel wall and platelet. In the setting of coronary stenoses, rupture of atheromatous plaque in relatively mildly stenosed vessels and subsequent thrombus formation underlie the majority of acute coronary syndromes. Vessel injury caused by plaque rupture exposes collagen and von Willebrand factor to platelets.2 Platelets then adhere, and local platelet activation stimulates further thrombus formation and additional platelet recruitment by supporting cell-surface thrombin formation and releasing adenosine diphosphate (ADP), serotonin, and thromboxane A2 (TXA2).3 Thrombus forms as platelets aggregate via the binding of bivalent fibrinogen to the glycoprotein (GP) IIb/IIIa complex. The pathways regulating platelet adhesion, activation, aggregation, and recruitment will be described in further detail below.

Platelets share many of the same biological mechanisms as other cells, including cytoskeletal structure, housekeeping enzymes, and signal transduction components; however, unlike most cells, platelets lack a nucleus and are unable to adapt to changing biological settings by altered transcription. Although platelets maintain some protein synthetic capacity from megakaryocyte-derived mRNA, most molecules needed to respond to various physiological and pathological stimuli are present in storage granules and membranes. Because the primary function of platelets is regulation of hemostasis, the main receptors play a direct role in the processes regulating adhesion or thrombus formation.

Advances in understanding the mechanisms governing thrombosis have led to the availability of new classes of antithrombotic drugs. Initially, characterization of arachidonic acid metabolism in platelets furthered an understanding of the therapeutic utility of cyclooxygenase inhibitors in vascular disease, most notably aspirin. The discovery and characterization of platelet receptors, such as the ADP receptor and the GP IIb/IIIa complex, have been associated with the development of novel classes of antiplatelet drugs, including thienopyridine derivatives and GP IIb/IIIa receptor antagonists. Further knowledge of receptors, their inhibitors, and signaling pathways, as well as an understanding of the interaction of thrombosis, inflammation, and the vessel wall, will continue to advance our understanding of thrombosis and enhance the development of new therapeutic interventions.

Platelet Adhesion: Initiation of the Thrombotic Cascade

The process of platelet activation, aggregation, and subsequent thrombus formation is frequently initiated by platelet
Platelet receptors and activation

Platelet activity is controlled by a variety of surface receptors that regulate various functions. Platelet receptors are affected by a wide variety of agonists (stimulants) and adhesive proteins. In general terms, the stimulation of platelet receptors triggers 2 distinct processes: (1) stimulation of various internal signaling pathways that lead to further platelet activation and granule release and (2) the capacity of the platelet to bind to other adhesive proteins/platelets, leading to the formation of a thrombus. Because both of these processes may cause thrombosis and vessel occlusion, many of these receptors have been and are being developed as targets for preventing clot formation. Because there are many families and subfamilies of receptors found on platelets that regulate a variety of platelet functions, only the main ones with current therapeutic relevance will be considered below.

Transmembrane receptors

The 7-transmembrane receptor family is the main agonist-stimulated receptor family. There are several 7-transmembrane receptors found on platelets, including the thrombin receptor, ADP receptors, prostaglandin receptors, lipid receptors, and chemokine receptors.

Thrombin receptor

Thrombin receptor is the major 7-transmembrane receptor found on platelets. The first identified was the protease activation receptor-1 (PAR1). The PAR class of receptors has a distinct mechanism of activation that involves specific cleavage of the N-terminus that acts as a ligand to the receptor. Other PAR receptors are present on platelets, including PAR2 (not activated by thrombin) and PAR4.

Figure 1. Platelet adhesion and aggregation. Damage or vascular injury exposes subendothelial von Willebrand factor (VWF) or collagen to the circulating blood. Platelets adhere to the site of injury when GP Ib/IX/V complex expressed on the platelet surface binds to the subendothelial VWF. This event triggers the synthesis and release of TXA2, serotonin, and ADP and causes activation of various platelet receptors (R). These events cause a conformational change in GP IIb/IIIa, enabling the high-affinity binding of fibrinogen and resulting in thrombus formation. This process can be attenuated by endothelial or platelet release of NO.

adhesion to the damaged vessel wall. The subendothelial components responsible for triggering platelet reactivity include different types of collagen, von Willebrand factor, fibronectin, and other adhesive proteins such as vitronectin and thrombospondin. The specific hemostatic response is dependent on the extent of damage, the specific matrix proteins exposed, and the determinants of blood flow. Several collagen-binding proteins that are expressed on the platelet surface also regulate collagen-induced platelet adhesion, specifically under flow conditions. These receptors include GP IV, GP VI, and integrin αβ₃. These receptors may interact in a synergistic way dependent on the flow conditions and relative exposure of distinct types of collagen or extracellular matrix proteins. The platelet-expressed GP Ib/IX/V complex adhesive receptor is central to both platelet adhesion and activation. Damage to the blood vessel wall exposes subendothelial von Willebrand factor and collagen to the circulating blood. This form of vascular injury occurs in the microcirculation and stenosed arteries, and the GP Ib/IX/V complex expressed on the platelet surface binds to the exposed von Willebrand factor, causing platelets to adhere to the subendothelium at the site of injury (Figure 1). In addition to anchoring the platelet to the injured vessel wall, engagement of the GP Ib/IX/V complex leads to the transduction of signals contributing to platelet activation. Von Willebrand factor–bound GP Ib/IX/V induces a conformational change in the GP IIb/IIIa receptor, transforming it from an inactive low-affinity state to an active receptor that binds additional von Willebrand factor or fibrinogen with high affinity.

Given the central role of the GP Ib/IX/V complex in platelet adhesion and aggregation, it has become an attractive target for the development of antiplatelet drugs. Therapeutics have been developed that target the interaction between the GP Ib/IX/V complex and von Willebrand factor as a way of preventing arterial thrombosis, including anti–von Willebrand factor monoclonal antibodies and the GP Ib/IX/V antagonists isolated from snake venoms. Another study examined the antithrombotic efficacy of the Fab fragments of the murine monoclonal antibody, 6B4, raised against the GP Ib/V/IX receptor of human platelets that significantly inhibited thrombus generation. The use of a monoclonal antibody to von Willebrand factor in a monkey model demonstrated sustained antiplatelet effect without extensive prolongation of the bleeding time. Currently, there are no published trials demonstrating clinical benefit with inhibition of GP Ib, and clinical success will depend on minimization of the presumed enhanced bleeding that may be seen as a result of inhibition of platelet adherence.
Specific inhibitors of PAR1 and PAR4 are undergoing study for potential future clinical use (Table 1).

**ADP Receptors**

With the success of the adenosine receptor inhibitors in the treatment of stroke and prevention of thrombosis after cardiac intervention, there has been an interest in these receptor-mediated pathways. Erythrocytes and endothelial cells secrete ADP, which contributes to hemostasis, thrombus formation, and vascular occlusion by stimulating platelet aggregation. In addition, the ADP secreted from the dense granules of stimulated platelets acts to potentiate platelet aggregation. In the platelet, ADP induces a rapid influx of external calcium, mobilizes calcium from intracellular stores, and attenuates the inhibition of adenylyl cyclase.

Transduction of ADP-induced signaling events involves the binding of ADP to purinergic receptors on the platelet surface. There are several distinct ADP receptors, classified as P2X<sub>1</sub>, P2Y<sub>1</sub>, and P2Y<sub>12</sub> (Figure 2). The P2X<sub>1</sub> receptor, a ligand-gated ion channel, mediates rapid, transient calcium influx and does not appear to play a role in platelet aggregation. ADP stimulation of the P2Y<sub>1</sub> G<sub>i</sub>-coupled receptor activates phospholipase C, causing internal calcium mobilization. Activation of the P2Y<sub>1</sub> receptor is thought to be responsible for mediating ADP-induced platelet shape change in addition to ADP-induced platelet aggregation. Additional evidence to support the role of the P2Y<sub>1</sub> receptor in platelet aggregation comes from studies of platelets from P2Y<sub>1</sub>-null mice that fail to aggregate in response to ADP.

The activation of both the P2Y<sub>12</sub> and P2Y<sub>1</sub> receptors is essential for ADP-induced platelet aggregation. The activation of the platelet P2Y<sub>1</sub> receptor mediates the initial rapid response to ADP, determining the maximal rate of ADP-induced platelet aggregation. The degree of sustained platelet aggregation, however, is believed to be due to ADP activation of the P2Y<sub>12</sub> receptor. Selective antagonism of the P2Y<sub>12</sub> receptor demonstrates that the P2Y<sub>12</sub> receptor plays a role in platelet aggregation.

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**Table 1. Platelet Inhibitors and Their Mechanism of Action**

<table>
<thead>
<tr>
<th>Platelet Inhibitors</th>
<th>Mechanism of Action</th>
<th>Alternative Mechanisms of Action</th>
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<tbody>
<tr>
<td><strong>Currently available</strong></td>
<td></td>
<td></td>
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<tr>
<td>Aspirin</td>
<td>Cyclooxygenase inhibitor</td>
<td>Antioxidant</td>
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<tr>
<td>Abciximab</td>
<td>GP Ib/IIa antagonist</td>
<td>CD11/CD18</td>
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<tr>
<td>Eptifibatide</td>
<td>GP Ib/IIa antagonist</td>
<td></td>
</tr>
<tr>
<td>Tiroffiban</td>
<td>GP Ib/IIa antagonist</td>
<td></td>
</tr>
<tr>
<td>Oral agents</td>
<td>GP Ib/IIa antagonist</td>
<td></td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>ADP receptor inhibitor</td>
<td></td>
</tr>
<tr>
<td>Ticlopidine</td>
<td>ADP receptor inhibitor</td>
<td></td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>Increases adenosine</td>
<td>inhibits cAMP and cGMP phosphodiesterases; increases PGI&lt;sub&gt;2&lt;/sub&gt;; antioxidant</td>
</tr>
<tr>
<td><strong>Under development</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitroaspirin (NCX4016)</td>
<td>NO donor&lt;sup&gt;04&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>P2Y12 antagonist (AR-C69931 MX)</td>
<td>Purinergic receptor inhibitor&lt;sup&gt;05&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>VWF-GP Ib/X inhibitors</td>
<td>Inhibit platelet adhesion</td>
<td></td>
</tr>
<tr>
<td>Collagen–GP VI inhibitors</td>
<td>Inhibit platelet adhesion</td>
<td>Inhibit aggregation</td>
</tr>
<tr>
<td>Thrombin–platelet inhibitors</td>
<td>Inhibit PAR1 receptors</td>
<td>Inhibit PAR4</td>
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</tbody>
</table>

VWF indicates von Willebrand factor.
Integrins

Integrins are adhesive and signaling molecules that consist of noncovalently associated heterodimers of α- and β-subunits. They normally exist in either a low- or high-affinity state that is altered by cytoplasmic signaling and phosphorylation of their cytoplasmic domains. Platelets possess 3 families of integrins, and only those present in abundant amounts and with current therapeutic relevance will be discussed.

Glycoprotein IIb/IIIa (αIIbβ3)

Although platelets lack a nucleus, they possess many of the signaling pathways found in nucleated cells. The activation of platelets results in a rapid series of a variety of signal transduction events, including tyrosine kinase activation, serine/threonine kinase activation, and lipid kinase activities. In unstimulated platelets, the major platelet integrin GP IIb/IIIa is maintained in an inactive conformation and functions as a low-affinity adhesion receptor for fibrinogen. This integrin is unique in that it is only expressed on platelets. After stimulation, the interaction between fibrinogen and GP IIb/IIIa forms intracellular bridges between platelets, leading to platelet aggregation (Figure 1). A conformational change in the extracellular domain of GP IIb/IIIa enables the high-affinity binding of soluble plasma fibrinogen as a result of a complex network of inside-out signaling events. This primary, reversible phase of platelet aggregation is precipitated by a series of extremely rapid and complex signaling pathways.

The GP IIb/IIIa receptor serves as a bidirectional conduit, with GP IIb/IIIa–mediated signaling (outside-in signaling) occurring immediately after the binding of fibrinogen and initiating intracellular signaling that further stabilizes the aggregate (Figure 3). The initial phase of outside-in signaling contributes to further activate the integrin GP IIb/IIIa via integrin clustering and the formation of a complex network of signaling and structural cytoskeletal proteins. Calcium mobilization, tyrosine phosphorylation, activation of phosphoinositide metabolism, and cytoskeletal reorganization result from the activation of the GP IIb/IIIa complex. This series of events transforms platelet aggregation from a reversible to an irreversible process.

Although aspirin and clopidogrel are effective antiplatelet agents, they are relatively weak antiaggregatory drugs. Drugs that specifically target the GP IIb/IIIa receptor and prevent the binding of fibrinogen inhibit this final common pathway for platelet aggregation. Platelet aggregation is completely inhibited by blockade of 80% of the surface GP IIb/IIIa receptors, and antagonism of the GP IIb/IIIa receptor inhibits platelet aggregation irrespective of the platelet activator. Multiple clinical trials have shown inhibition of GP IIb/IIIa to be a highly effective antithrombotic strategy. Currently, there are several GP IIb/IIIa inhibitors in clinical use (Table 1). Abciximab is a Fab fragment of the 7E3 antibody that has high affinity and a slow rate of dissociation from GP IIb/IIIa. In contrast, the small molecules eptifibatide and tirofiban have a much more rapid rate of dissociation.

Several ligands of integrins, including fibrinogen and von Willebrand factor, possess 1 of the 2 amino acid sequences 95 to 97 (Arg-Gly-Asp or RGD) and 572 to 575 (Arg-Gly-Asp-Ser or RGDSD) that enable them to bind to the activated GP IIb/IIIa receptor and inhibit platelet aggregation. These nonpeptide mimetics are unique in that they have the potential for oral administration that initially was thought to be useful for long-term antiplatelet therapy; however, several oral GP IIb/IIIa inhibitors have been evaluated, and their use has not been associated with clinical benefit. Reasons for this discrepancy are unclear, and potential explanations have...
Platelet Secretion and Recruitment

After stimulation and aggregation, platelets release various substances that lead to activation of additional circulating platelets. These platelets also become activated, and, by fibrinogen bridging with the adherent platelets, a thrombus is formed. This process of attracting additional platelets is termed recruitment. As with activation and aggregation, the process of recruitment is a balance between prothrombotic and antithrombotic forces (Figure 1). Platelets release the contents of their intracellular granules in response to activation. Impairment of this process affects not only normal hemostasis and thrombosis but vascular remodeling. The secretory granules contain coagulation proteins, adhesion molecules, cytokines, integrins, growth factors, and inflammatory modulators.

One of the best-characterized substances released by platelets that lead to recruitment is thromboxane. Not preformed in a granule, it is generated on platelet stimulation. After platelet activation, arachidonic acid is liberated from membrane phospholipids by phospholipase A_2 and C. The catalytic activities of prostaglandin (PGH)-synthase sequentially metabolize arachidonic acid to generate prostaglandins. Aspirin inhibits the cyclooxygenase activity of PGH-synthase, which in turn blocks the metabolism of arachidonic acid to prostaglandin \( H_2 \) (PGH\(_2\)), the precursor of \( TXA_2 \) and other cyclic prostanooids (prostacyclin and other prostaglandins).

Platelets synthesize and release \( TXA_2 \) on stimulation with the agonists collagen, thrombin, or ADP. Engagement of the platelet \( TXA_2 \) receptor activates phospholipase C and liberates intracellular calcium. The increase in intracellular calcium amplifies platelet stimulation and results in the synthesis and release of additional ADP and \( TXA_2 \) from activated platelets. Both ADP and \( TXA_2 \) participate in a positive feedback loop that leads to platelet aggregation and recruitment. In both normal subjects and patients with atherosclerotic vascular disease, aspirin produces a dose-dependent inhibition of platelet cyclooxygenase activity after the administration of a single oral dose. Owing to the irreversible inhibition of cyclooxygenase by aspirin and the inability of platelets to synthesize new protein, the effect of aspirin is maintained for the life span of the platelet (7 to 10 days). Cyclooxygenase activity returns only as new platelets are formed and released into the circulation. The inhibitory effects of aspirin are pronounced when relatively weak platelet agonists are used but are less pronounced against stronger agonists, such as thrombin, that can induce platelet activation in the absence of \( TXA_2 \). Importantly, the majority of platelet responses remain unaffected by aspirin treatment. Aspirin does not inhibit shear stress–induced platelet activation and platelet adhesion.

Clinical trials have demonstrated the efficacy of aspirin in both primary and secondary prevention of myocardial infarction, stroke, and cardiovascular death. Despite proven benefit, the absolute risk of recurrent vascular events among patients taking aspirin remains relatively high, an estimated 8% to 18% after 2 years. Therapeutic resistance to aspirin might explain a portion of this risk, although the mechanism for aspirin resistance is uncertain. The answer is likely a combination of clinical, biological, and genetic properties affecting platelet function. The redundancy of platelet activation pathways and receptors may contribute to the problem of aspirin resistance, as may compliance or effects of increased shear stress. In addition, epinephrine is known to enhance platelet activation, and elevated catecholamines found in patients with acute coronary syndromes may render them resistant to the effects of aspirin.

Substances such as thromboxane and ADP may recruit additional platelets to a growing thrombus; this process is self-limited in most clinical situations. As each platelet activates and adheres, it releases these prothrombotic substances; however, mechanisms must exist to limit or prevent the process of thrombus growth before vessel occlusion. Although rheological forces within the vessel are important, platelets also release antithrombotic substances that may provide negative feedback for thrombus formation.

One such substance is nitric oxide (NO). NO is known to be released by the intact endothelium preventing adherence of platelets to the vessel wall under normal conditions (Figure 1). NO inhibits platelet activation, prevents thrombosis, and inhibits the normal activation-dependent increase in the expression of platelet surface GPs, including P-selectin and the integrin GP IIb/IIIa complex. NO inhibits platelet function by stimulating soluble guanylyl cyclase to produce cGMP. This action results in the stimulation of cGMP-dependent protein kinase, leading to a reduction in fibrinogen binding to GP IIb/IIIa and modulation of phospholipase A_2 and C–mediated responses. NO decreases the oxidation of arachidonate and inhibits the agonist-dependent increase in platelet cytosolic-free calcium in a cGMP-dependent and -independent manner. NO released specifically from platelets inhibits platelet recruitment to a growing thrombus (Figure 1) and activates platelets from patients with acute coronary syndromes produce significantly less NO than that in patients with stable coronary artery disease.

Activation and recruitment of platelets are tightly regulated. Normally, adhesion of platelets to the endothelium and platelet activation are prevented by several mechanisms, including endothelial cell production of prostacyclin, NO, and ecto-ADPase. Prostacyclin, released by the vessel wall, inhibits platelet function via cAMP, and its effects are also mediated by high-affinity prostanooid receptors found on the platelet. CD39 is a major ecto-ADPase (also known as nucleoside triphosphate diphosphohydrolase [NTPDase]-1) and is the main vascular NTPDase that converts ATP and ADP to AMP. By converting ADP to AMP, CD39 suppresses platelet activation and platelet–endothelial cell interactions.

Thrombus Stability and Disaggregation

The final phase of thrombus formation occurs after the rapid signals arising from G protein–coupled receptors have oc-
curved and the receptors have been desensitized. This phase serves to stabilize the platelet thrombus and prevent premature disaggregation. Although less is known about this phase, it appears that outside-in signaling events regulated through cell-surface integrins and receptor tyrosine kinase signals are primary mediators.

Although much is known about the normal activation of platelets, there have been few observations demonstrating reversibility of the aggregation process. The use of fibrinolytic strategies is clearly established for the dissolution of fibrin thrombus, but the disengagement of fibrinogen from the platelet has not been a common therapeutic target. GP IIb/IIIa inhibitors have been shown to promote dissolution of platelet-rich thrombi, but these in vitro observations required relatively high concentrations of GP IIb/IIIa inhibitors, suggesting that such treatment may only be feasible with the use of local delivery. Although phosphoinositide kinase (PI3-kinase) does not appear to be essential in the initial activation of GP IIb/IIIa, the sustained activation and thus maintenance of aggregation may be due to the generation of the PI3-kinase product PtdIns(3,4)P2 after GP IIb/IIIa engagement. Therefore, the activation of PI3-kinase is critical for strengthening platelet aggregation.

The effects of NO on platelet function are similar to the effects of PI3-kinase inhibition. Moreover, NO donors are able to reverse GP IIb/IIIa activation, suggesting that NO plays a consequential role in mediating platelet aggregation. Inhibition of PI3-kinase and the addition of exogenous NO donors appear to have additive platelet inhibitory effects; however, the relevance of these findings to endogenous NO release is unclear. Superoxide generated by stimulated platelets can increase both platelet adhesion and aggregation, presumably as a consequence of its reaction with NO and the attenuation of NO bioactivity. PI3-kinase plays a role in regulating NADPH oxidase–generated superoxide in platelets, altering the bioactivity of platelet NO that contributes to platelet disaggregation. PI3-kinase inhibition causes progressive reversal of aggregation after initial aggregation. The ability of NO donors to induce platelet disaggregation has also been linked to the diminished activation of GP IIb/IIIa and fibrinogen binding. The prolonged activation of GP IIb/IIIa appears essential for irreversible aggregation.

Genetic Variants and Arterial Thrombosis

The best-characterized genetic variants associated with thrombosis have been those effecting the coagulation and fibrinolytic cascades. In these settings, genetic alteration of blood components may lead to enhanced thrombosis, specifically related to deep venous thrombosis and pulmonary embolism. However, many reports of polymorphisms have been association studies that are prone to error and have had inconstant outcome. Unlike venous thrombosis, the search for relevant genetic variants in arterial thrombosis has been less successful. Likely, this is because atherothrombosis is a complex and highly prevalent polygenic disease, and the phenotype is rarely due to an isolated genetic abnormality or variant.

Through the recognition of familial tendencies in venous thrombosis, genetic abnormalities in the coagulation system have been found. Those initially described were mutations that led to a loss of function of endogenous anticoagulants, including antithrombin III, protein C, or protein S (Table 2). Other mutations have been associated with enhanced thrombotic function such as factor V Leiden or increased coagulation factors (prothrombin G20210A). Alterations of the fibrinolytic cascade have also been linked with changes in thrombosis but have been complicated by the presence of numerous mutations, which has made genetic testing difficult. These variants have included those of tissue plasminogen activator.

Although the vast number of polymorphisms studied have been factors in the coagulation cascade and are associated with venous thrombotic disease, numerous other associations have been reported that may potentially alter arterial thrombosis. This has included abnormalities in homocysteine metabolism and platelet surface GPs (Table 2). The GP VI gene is polymorphic with several identified variants, and the T13254C polymorphism specifically leads to an amino acid substitution (serine to proline) and has been associated with increased risk of myocardial infarction; however, the functional effects of many of these polymorphisms have not been clearly ascertained. A large, population-based study suggested that heritable factors play a major role in determining platelet aggregation compared with environmental covariates. The study also reported that the platelet GP IIIa PI and fibrinogen HinfIII polymorphisms contributed <1% to the overall variance. This study concluded that there is a paucity of data with regard to the genetic epidemiology of abnormal platelet aggregability and that additional studies are needed to identify key genetic variants regulating platelet aggregation.

<table>
<thead>
<tr>
<th>TABLE 2. Genetic Variants Associated With Thrombosis</th>
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<tbody>
<tr>
<td>Procoagulant proteins</td>
</tr>
<tr>
<td>Fibrinogen</td>
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<tr>
<td>Prothrombin (20210G→A)</td>
</tr>
<tr>
<td>Factor VII</td>
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<tr>
<td>Protein C anticoagulant pathway</td>
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<tr>
<td>Factor V Leiden: 1691G→A (Arg506Gln)</td>
</tr>
<tr>
<td>Thrombomodulin 1481C→T (Ala455Val)</td>
</tr>
<tr>
<td>Fibrinolytic proteins with known polymorphisms (multiple)</td>
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<tr>
<td>Tissue plasminogen activator</td>
</tr>
<tr>
<td>Plasminogen deficiency</td>
</tr>
<tr>
<td>Dystifibrinogenemia</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor</td>
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<td>Homocysteine</td>
</tr>
<tr>
<td>Cystathionine β-synthase 833T→C</td>
</tr>
<tr>
<td>5,10-Methylene tetrahydrofolate reductase 677C→T</td>
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<tr>
<td>Platelet receptors</td>
</tr>
<tr>
<td>GP VI T13254C polymorphism</td>
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<tr>
<td>GP IIIa</td>
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<tr>
<td>GP Ib</td>
</tr>
<tr>
<td>Thrombin receptor PAR1–5061→D</td>
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</tbody>
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Polymorphisms in platelet surface GPs have been identified, including the (A1/2) polymorphism resulting in a conformational change at the amino terminus of the β-3 chain of the platelet fibrinogen receptor GP IIb/IIIa and polymorphisms in the platelet collagen (GP Ia/IIa and GP VI) and von Willebrand receptors (GP Ib/IX). Mutations have also been identified in other platelet surface receptors including G(i)-linked platelet ADP receptor P2Y. The relevance of these polymorphisms to their relevant class of antithrombotic drug is not fully known at this time.

Other polymorphisms in thrombosis have been examined, including those of the endothelial NO synthase (eNOS) gene. Several polymorphic variants of the eNOS gene have been described, and the most extensively characterized variant is the 894-G/T polymorphism in exon 7 of the gene, resulting in a glutamate or aspartate at position 298. The 894T allele is associated with higher plasma levels of NOs in healthy individuals. Epidemiological studies have shown an increased risk of hypertension, myocardial infarction, and stroke in patients homozygous for the Glu298Asp variant.

Another eNOS polymorphism, designated ecNOS4a, has been identified on intron 4 and has been associated with premature coronary artery disease and also with a history of myocardial infarction. Polymorphisms in the promoter region and exon 7 have been associated with lower levels of platelet-derived NO.

Other genetic abnormalities that may regulate thrombosis are polymorphisms of glutathione peroxidase. Glutathione peroxidase is a selenium-containing enzyme, and selenium deficiency has been reported in patients with acute myocardial infarction and coronary atherothrombotic disease. Glutathione peroxidase potentiates the inhibition of platelet function by NO by decreasing LOOH concentrations. Impairment of this process can lead to a clinical thrombotic disorder, and a similar deficiency has been reported in 5 other families with childhood stroke.

**Contribution of Inflammation to Thrombosis**

Accumulating evidence suggests that inflammation plays an important role during the acute thrombotic phase of unstable coronary syndromes. Recent data demonstrate that patients with acute coronary syndromes not only have increased interactions between platelets (homotypic aggregates) but also have increased interactions between platelets and leukocytes (heterotypic aggregates) detectable in circulating blood. These aggregates form when platelets are activated and undergo degranulation, after which they adhere to circulating leukocytes; accumulating information suggests that these events contribute to the atherothrombotic process. Platelets bind via P-selectin (CD62P) expressed on the surface of activated platelets to the leukocyte receptor, P-selectin GP ligand-1 (PSGL-1). This association leads to increased expression of CD11b/CD18 (Mac-1) on leukocytes, which itself supports interactions with platelets, partially via bivalent fibrinogen linking this integrin with its platelet surface counterpart, GP IIb/IIIa.

The binding of platelets and leukocytes in acute coronary syndromes highlights the interaction between inflammation and thrombosis in atherothrombotic disease. Plaque rupture promotes activation of the inflammatory response, and increased expression of tissue factor initiates extrinsic coagulation. The expression of tissue factor on both endothelial cells and monocytes is partially regulated by proinflammatory cytokines, including tumor necrosis factor and interleukin-1. In addition to initiating coagulation, tissue factor interacts with P-selectin, accelerating fibrin formation and deposition. Platelet surface P-selectin also induces the expression of tissue factor on monocytes, enhances monocyte cytokine expression, and promotes CD11b/CD18 expression. This prothrombotic process is regulated by production of endothelium-derived NO, which reduces endotoxin- and cytokine-induced expression of tissue factor.

In addition to the formation of platelet-monocyte aggregates, measurement of the cytokine, soluble CD40 ligand, also reflects the interface between thrombosis and inflammation. The CD40 ligand is a trimeric, transmembrane protein of the tumor necrosis factor family and, with its receptor CD40, is an important contributor to the inflammatory processes leading to atherosclerosis and thrombosis. Many immune and vascular cells have been found to express CD40 and/or CD40 ligand, and both have been clearly shown to be present in human atheroma. In platelets, CD40 ligand is rapidly translocated to the surface after stimulation and is upregulated in fresh thrombus. The surface-expressed CD40 ligand is then cleaved from the platelet over a period of minutes to hours, subsequently generating a soluble fragment (soluble CD40 ligand). Although also shed from stimulated lymphocytes, it is estimated that >95% of the circulating CD40 ligand is derived from platelets. Soluble CD40 ligand has been shown to be associated with increased cardiovascular risk in apparently healthy women. Soluble CD40 ligand can identify patients at high risk of acute coronary syndromes, and this increased risk associated with elevated soluble CD40 ligand levels is reduced by abciximab treatment. These observations suggest that soluble CD40 ligand can be used to identify patients with enhanced thrombotic risk.

Soluble CD40 ligand has also been implicated in the acute thrombotic process. On endothelial cells or monocytes, the engagement of CD40 with CD40 ligand leads to the synthesis of adhesion molecules, chemokines, and tissue factor and causes the activation of matrix metalloproteinases that are known to contribute to atherothrombotic pathophysiology. Soluble CD40 ligand has a lysine-arginine-glutamic acid (KGD) motif that also allows for its binding to the platelet GP IIb/IIIa complex. It is possible that such binding is blocked in the setting of GP IIb/IIIa inhibitors, potentially altering the clot-stabilizing properties of soluble CD40 ligand.

There is also evidence that inflammation leads the activation of coagulation with subsequent fibrin deposition. Expression of procoagulant mediators such as tissue factor can initiate activation of the coagulation cascade, and the thrombin subsequently generated activates platelets and fibrinogen. The effect of inflammation may also occur as a result of physiological anticoagulant pathways. The interaction between inflammation, coagulation, and fibrinolysis has been recently reviewed by Levi and colleagues.
Conclusion

It is essential to further our understanding of the thrombotic pathway to continue to develop novel ways of controlling these processes in vivo. An important lesson that has emerged from numerous trials is that increased antithrombotic potency may not guarantee enhanced clinical benefit. Whether this is due to problems with the specific antithrombotic agents or the inherent limitations of our current means of measuring thrombosis is not known. Novel therapies are also being developed to target the redundant pathways of platelet adhesion, activation, and aggregation. These include (1) new inhibitors of the P2Y1 and P2Y12 receptors; (2) the CD40-CD40 ligand system; (3) RANTES; (4) P-selectin; (5) CD39/NTTDPase; (6) the GP Ib/V/IX complex–von Willebrand factor; and (7) protease-activated receptors. Other specific targets are listed in Table 1, including anti–GP VI therapy and NO-releasing aspirin derivatives. Efforts are also ongoing to enhance implementation of existent therapy, to target therapy selectively to high-risk patients and to those likely to respond (pharmacogenomics), and to study the incremental benefits and safety of various antiplatelet combinations and their interaction with other medications. Continuing advances in understanding the mechanisms of thrombosis, as well as the development of new techniques for studying its regulation, have led to a clearer understanding of thrombotic disease as well as the availability of new classes of antithrombotic drugs. Because the coagulation and platelet-dependent pathways are also intimately tied to the atherosclerotic process, future understanding may also affect the global and chronic state of the vasculature and further protect vessel patency.

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