Molecular Regulation of Platelet-Dependent Thrombosis

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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...
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.

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.
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 P2X1, P2Y1, and P2Y12 (Figure 2). The P2X1 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 P2Y1 receptor activates phospholipase C, causing internal calcium mobilization. Activation of the P2Y1 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 P2Y1 receptor in platelet aggregation comes from studies of platelets from P2Y1-null mice that fail to aggregate in response to ADP.

The activation of both the P2Y12 and P2Y1 receptors is essential for ADP-induced platelet aggregation. Selective antagonism of the P2Y12 receptor demonstrates that the P2Y12 receptor plays a role in platelet aggregation. Under development agents are also being studied to target ADP receptors.
a role in sustaining and amplifying ADP-induced aggregation.22

The thienopyridine derivatives ticlopidine and clopidogrel are clinically utilized inhibitors of ADP-induced platelet aggregation (Table 1). The antiplatelet effect of thienopyridine derivatives is due to irreversible inhibition of ADP binding to platelet purinergic receptors.23,24 Metabolism of clopidogrel by the hepatic cytochrome P450-1A enzyme system to an active metabolite23,24 is essential for its in vivo antiplatelet effects.23,24 This metabolite of clopidogrel displays the same in vivo effects as seen in vitro, such as inhibition of adenylyl cyclase and inhibition of ADP-induced aggregation.23,24 Both drugs are mechanistically and structurally similar; however, owing to a higher incidence of severe neutropenia and other adverse effects associated with ticlopidine, clopidogrel has become the primary thienopyridine derivative used in clinical settings.25

**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 (α\text{IIb}\beta\text{IIIa})**

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.26 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.27 This primary, reversible phase of platelet aggregation is precipitated by a series of extremely rapid and complex signaling pathways.28

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.29 Platelet aggregation is completely inhibited by blockade of 80% of the surface GP IIb/IIIa receptors,30 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.31 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 epifibatide 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 RGDS) that enable them to bind to the activated GP IIb/IIIa receptor and inhibit platelet aggregation.32 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.33–36 Reasons for this discrepancy are unclear, and potential explanations have
included hypothetical partial agonist activity, a prothrombotic effect of the oral compounds, or a direct toxic effect that is unrelated to platelet activation, such as apoptosis.37–39 Additionally, it has been observed that some oral GP IIb/IIIa inhibitors augment small platelet microaggregate formation after stimulation.38,39

**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 A₂ and C. The catalytic activities of prostaglandin (PG)H-synthase sequentially metabolize arachidonic acid to generate prostaglandins.40 Aspirin inhibits the cyclooxygenase activity of PGH-synthase, which in turn blocks the metabolism of arachidonic acid to prostaglandin H₂ (PGH₂), the precursor of TXA₂ and other cyclic prostanooids (prostacyclin and other prostaglandins).

Platelets synthesize and release TXA₂ on stimulation with the agonists collagen, thrombin, or ADP.41,42 The TXA₂ released by platelets then binds to platelet thromboxane receptors.41,42 Engagement of the platelet TXA₂ 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 TXA₂ from activated platelets. Both ADP and TXA₂ 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 TXA₂. Importantly, the majority of platelet responses remain unaffected by aspirin treatment. Aspirin does not inhibit shear stress–induced platelet activation and platelet adhesion.43

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 mechanisms for aspirin resistance are 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.43,45 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.45

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,46,47 prevents thrombosis,48 and inhibits the normal activation-dependent increase in the expression of platelet surface GPs, including P-selectin and the integrin GP IIb/IIIa complex.49 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 A₂– and C–mediated responses.50 NO decreases the oxidation of arachidonate51 and inhibits the agonist-dependent increase in platelet cytosolic-free calcium in a cGMP-dependent52 and -independent manner.53 NO released specifically from platelets inhibits platelet recruitment to a growing thrombus (Figure 1), and activated platelets from patients with acute coronary syndromes produce significantly less NO than that in patients with stable coronary artery disease.55

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.56,57 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.58 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.59

**Thrombus Stability and Disaggregation**

The final phase of thrombus formation occurs after the rapid signals arising from G protein–coupled receptors have oc-
curried and the receptors have been desensitized. This phase 
serves to stabilize the platelet thrombus and prevent prema-
ture 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 fibrino-
lytic 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 
(P13-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 engage-
ment. 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 plate-
lets can increase both platelet adhesion and aggregation, 
possibly 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 plate-
lets, altering the bioactivity of platelet NO that contributes to 
platelet disaggregation. PI3-kinase inhibition causes pro-
gressive 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 activation 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, specif-
ically 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.

**TABLE 2. Genetic Variants Associated With Thrombosis**

<table>
<thead>
<tr>
<th>Procoagulant proteins</th>
<th>Fibrinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prothrombin (20210G→A)</td>
<td>Factor VII</td>
</tr>
<tr>
<td>Protein C anticoagulant pathway</td>
<td>Factor V Leiden: 1691G→A (Arg506Gln)</td>
</tr>
<tr>
<td>Thrombomodulin 1481C→T (Ala455Val)</td>
<td>Fibrinolytic proteins with known polymorphisms (multiple)</td>
</tr>
<tr>
<td>Tissue plasminogen activator</td>
<td>Plasminogen deficiency</td>
</tr>
<tr>
<td>Dystifibrinogenemia</td>
<td>Plasminogen activator inhibitor</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>Cystathionine β-synthase 833T→C</td>
</tr>
<tr>
<td>S,10-Methylene tetrahydrofolate reductase 677C→T</td>
<td></td>
</tr>
<tr>
<td>Platelet receptors</td>
<td>GP VI T13254C polymorphism</td>
</tr>
<tr>
<td>GP IIIa</td>
<td>GP Ib</td>
</tr>
<tr>
<td>Thrombin receptor PAR1–5061→D</td>
<td></td>
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</tbody>
</table>

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 throm-
botic function such as factor V Leiden or increased coagula-
tion factors (prothrombin G20210A). Alterations of the fi-
brinolytic cascade have also been linked with changes in 
thrombosis but have been complicated by the presence of 
umerous mutations, which has made genetic testing diffi-
cult. These variants have included those of tissue plasmino-
gen 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 throm-
bus. This has included abnormalities in homocysteine me-
tabolism 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 func-
tional effects of many of these polymorphisms have not been 
clearly ascertained. A large, population-based study sug-
gested that heritable factors play a major role in determining 
platelet aggregation compared with environmental covari-
ates. The study also reported that the platelet GP IIIa PlA2 
and fibrinogen HindIII 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
function. Polymorphisms in platelet surface GPs have been identified, including the (A1/2) polymorphism resulting in conformational change at the amino terminus of the β-3 chain of the platelet fibrinogen receptor GP Ib/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 the 894-T 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 increases the 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 Ib/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 endothoxin- 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 immunologic 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 Ib/IIIa complex. It is possible that such binding is blocked in the setting of GP Ib/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/NTPase; (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 potency.

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