Platelets and Shear Stress

By Michael H. Kroll, J. David Hellums, Larry V. McIntire, Andrew I. Schafer, and Joel L. Moake

Clinical bleeding or thrombosis results from a disturbance in the balance between a complex network of procoagulant and anticoagulant factors. This network involves three primary interactions, first described by the eminent pathologist Rudolph Virchow during the previous century, between blood (soluble and cellular constituents), the blood vessel (including fixed and dynamic responses), and blood flow. This review examines one increasingly recognized feature of this interplay that appears to affect both hemostasis and thrombosis. This feature is the effect on blood platelets of the mechanical forces of shear generated by flowing blood. Knowledge of the link between platelets and shear stress provides clues to general mechanisms governing physiologic and pathologic processes by re-emphasizing the importance of physical forces in regulating cellular function in vivo.

Platelets have long been regarded as the preeminent cell involved in physiologic hemostasis and pathologic thrombosis. In both cases, the plasma protein von Willebrand factor (vWF) and the blood platelet work together to effect the biologic response. The versatility in biologic responses mediated by a single receptor-ligand coupling has been, and remains, one of the challenging conundrums placed before clinical investigators and practicing hematologists. It is all the more intriguing when one considers that mixing vWF with platelets in a static or stirred suspension evokes no response. The discovery that the mechanism of toxicity of the antibiotic ristocetin is its capacity to induce plasma vWF to bind to platelet glycoprotein (Gp) Ib stimulates numerous important discoveries about the structure and function of vWF and its platelet receptors, including the nosology of von Willebrand (vWD) disease. However, elucidation of mechanisms of hemostasis and thrombosis in vivo required the convergence of classical pathology with modern molecular biology. One hundred and forty years after it was proposed, Rudolf Virchow's concept of the pathogenesis of thrombosis holds the key to understanding the relationship between platelets and shear stress.

Virchow's triad is a concept that is old but does not recede; rather, it confronts observations and their interpretation within a context of the interrelated pathophysiologic elements of blood, blood vessel, and blood flow. It is the application of Virchow's principles to the puzzle of vWF-mediated platelet aggregation that ultimately led to the discovery that rheologic factors mediate the binding of vWF to platelets in vivo, and that shear stress appears to be the physiologic, or pathophysiologic, equivalent of ristocetin. This review will attempt to summarize our current understanding of the molecular basis of Virchow's triad operating in platelet-dependent arterial thrombosis, and to explore the mechanisms by which rheologic and vascular factors interact to effect platelet-dependent hemostasis.

The scope of the problem of arterial thrombosis is staggering: at least 5 million adults in the United States alone suffer from related symptoms. About 50% of the annual nonaccident deaths in the United States are caused by thrombi predominantly composed of platelets in coronary or cerebral arteries. In addition, thrombosis of these and the peripheral arteries causes extensive morbidity. Antiplatelet agents presently available in clinical practice, which were originally tested predominantly in static or stirred systems, are only moderately effective in preventing the development or recurrence of thrombotic diseases of the coronary and cerebrovascular circulation. To improve the therapy of arterial thrombotic disorders, therefore, it is imperative to establish basic mechanisms of platelet thrombus formation under conditions of arterial blood flow. In vitro systems capable of modeling flow-mediated platelet adhesion and aggregation have been developed to investigate the mechanisms by which mechanical forces affect platelet thrombus formation. The reconstitution in vitro of the components of Virchow's triad operating in vivo in pathologically constricted arteries is likely to provide improved methods for the evaluation of...

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new antithrombotic agents. Modern investigations of mechanisms of platelet adhesion and aggregation must go beyond static conditions and begin to account for rheologic variables that affect physiologic and pathologic responses.

**PRINCIPLES OF RHEOLOGY**

In the past several years, engineering principles have been used to design model systems that simulate flowing conditions in injured, narrowed, or branched arteries. These models are used to generate controlled mechanical forces in vitro similar to those produced by hemodynamic forces in vivo. The mechanical forces produced in blood vessels may result from (1) luminal pressure changes that cause blood flow (which yields shear stress), or (2) transmural pressure changes that cause circumferential deformation of the layers of the vessel wall during the cardiac cycle (caused by tensile stress). Fluid shear stress (in dynes per square centimeter) is the force per unit area generated by flow of a viscous liquid.

Tensile stress produces strain, which is a measure of the percent change in vessel circumference generated by the expansion and contraction of the luminal diameter. Strain is the force which would tear a cell, or layer, from its normal position within the vessel wall. One effect of tensile stress, and the strain it produces, is to stretch individual cells that are anchored.

The mechanical force most relevant to platelet-mediated hemostasis and thrombosis is shear stress. Shear stress is defined as 'the force per unit area between laminae'; and blood flow can be described as an ‘infinite number of infinitesimal laminae sliding across one another, each lamina suffering some frictional interaction with its neighbors.’ Liquid shear stress in a tubular blood vessel is the force per unit area applied to the blood within the flowing stream. Wall shear stress is the force per unit area applied by the flowing viscous blood to the vessel surface (Fig 1).

In the relatively high flow environment of the arterial circuit, blood is a suspension that approximately behaves as a Newtonian fluid. This means that shear stress (τ) in a tubular chamber having radius r and flow direction z can be represented by the mathematical formula: \( \tau = \frac{4 \mu Q}{\pi r^4} \), with \( \frac{dv_z}{dr} \) the local velocity gradient (or shear rate) and viscosity (\( \mu \)) the proportionality constant. This formula for shear stress holds throughout the fluid, including the immediate vicinity of the tube wall, where it yields the wall shear stress. In Fig 1, the shear rate \( \frac{dv_z}{dr} \) is designated as \( \gamma_z \). As illustrated in the figure, the shear rate (and hence, the shear stress) varies continuously through the fluid from zero at the centerline to a maximum at the wall. For a Newtonian fluid, the relationship between shear stress and shear rate \( \tau = -\mu \frac{dv_z}{dr} \) allows one to convert simply from shear stress to shear rate: shear rate in cm/s per cm or s\(^{-1}\) = shear stress/viscosity. For example, in whole blood with a viscosity of 0.038 poise, a wall shear stress \( \tau_w = 90 \) dynes/cm\(^2\) results in a wall shear rate of 2.368 s\(^{-1}\). Wall shear stress of Newtonian fluids for flow in tubular vessels can be calculated as a function of volumetric flow rate: \( \tau_w = 4 \mu Q / \pi r^4 \), where \( \mu \) is viscosity, \( Q \) is the volumetric flow rate, and \( r \) = radial distance of the tubular chamber. This formula has been used to calculate shear forces in vivo (Table 1).

Steady Newtonian flow through a cylinder generates a parabolic velocity profile and a linear distribution of shear stresses, with liquid components (e.g., platelets) at the periphery of the cylinder (the wall) subjected to maximal shear stress and liquid components at the center of the cylinder subjected to zero shear stress (Fig 1). Time-averaged blood flow through the arterial circuit is often modeled using this simplified approach, and wall shear stress data based on this model form the foundation for much of the information discussed in this review. However, blood flow in vivo is far more complicated, and rheologic variables related to eddy formation, pulsatility, and dynamic constriction may predominate under many physiologic and pathologic conditions.

Fluid shear stresses in the circulation are imposed on the surfaces of blood cells and blood vessels. Within a liquid stream, freely moving particles rotate in the shear field and are therefore subjected to time-varying (sinusoidal) shear stress. The time-average of this shear stress is the liquid shear stress applied by viscometers (see below). However, the time-average of shear stresses sensed by platelets is not identical to that sensed by an ideal spherical particle. This is because platelets have an irregular shape (unactivated platelets are discoid and activated platelets become rounded with many irregular tendrils) that creates a surface subjected to tangential shearing forces even near the center of a flowing

**Table 1. Typical Ranges of Wall Shear Rates and Wall Shear Stresses, Assuming That the Viscosity of Whole Blood Is 0.038 Poise**

<table>
<thead>
<tr>
<th>Blood Vessel</th>
<th>Wall Shear Rate ( \gamma_z )</th>
<th>Wall Shear Stress ( \tau_w ) (dynes/cm(^2))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large arteries</td>
<td>200-800</td>
<td>11.4-30.4</td>
</tr>
<tr>
<td>Arterioles</td>
<td>500-1,600</td>
<td>19.0-60.8</td>
</tr>
<tr>
<td>Veins</td>
<td>20-200</td>
<td>0.76-2.6</td>
</tr>
<tr>
<td>Stenotic vessels</td>
<td>800-10,000</td>
<td>30.4-380</td>
</tr>
</tbody>
</table>

stream at some distance from the vessel wall. The precise level of shear stress on platelets in flowing blood is not known. However, it is known that platelets in whole blood are pushed toward the periphery of flow nearer to the maximal shear stresses generated at the vessel wall.

It should also be emphasized that thrombus formation is a dynamic process, and the components of Virchow’s triad operating within the developing thrombus continually change over time. For example, suspended platelets passing through stenotic arteries are transiently subjected to elevated shear stresses, whereas adherent platelets and platelets accumulating in the developing thrombus are continuously subjected to elevated shear stresses until flow is decreased by the occlusive thrombus. Flow probes placed in arteries and arterioles in animal models of occlusive vascular disease have been used to provide data on the complex rheologic forces operating in vivo.

DEVELOPMENT OF THE HYPOTHESIS THAT SHEAR AFFECTS PLATELET FUNCTION

There was little research in arterial thrombosis and relatively little understanding of platelet function before 1960. In 1962, Born devised a simple method for studying the response of platelets to chemical agonists. The method involved recording changes in light transmission of a stirred platelet suspension as platelet aggregates are forming. This “platelet aggregometer” had a profound effect on the study of platelet reactions, and it rapidly came into routine and widespread use. Because platelets are stirred to maintain a suspension, platelet-platelet interactions in the platelet aggregometer take place under the influence of fluid mechanical shear stress. However, the shear stress is low and it varies throughout the platelet suspension in a complex and uncharacterized way. Therefore, the aggregometer is inadequate for studying platelet responses within the context of Virchow’s triad.

By the middle of the next decade, quantitative rheologic methods began to be used to study platelet reactions under controlled shear stress, and several workers established the importance of the shear field itself as a determinant of platelet reactions. In 1975 Brown et al. reported the direct effect of shear stress on platelets in a cone-plate viscometer. These experiments were undertaken within a research program to try to develop an artificial heart, and the hypothesis under investigation was that shear stress generated by intracardiac devices leads to mechanical platelet damage. These investigators observed that pathologic levels of shear stress (>50 dynes/cm²) applied to platelet-rich plasma (PRP) induced changes in platelet morphology, along with secretion and aggregation. The cause of these changes, vis a vis platelet activation versus platelet lysis, was uncertain, although lysis was minimal at shear stresses below 250 dynes/cm². In 1978 Weiss et al. reported the first experiments to demonstrate that platelet adhesion to a vascular surface is both shear rate and vWF-dependent. These investigators used a concentric cylinder flow system over everted rabbit aorta, and citrate- and non-anticoagulated blood from patients with vWD (in which the ligand, vWF, is deficient or defective) and Bernard-Soulier syndrome (in which the platelet GpIb/IX/V receptor complex is deficient). Shear forces at low (venous) levels had no measurable effect on platelets, but as the magnitude and duration of shear forces were increased, measurable changes developed in the three indices of platelet response (adhesion, secretion, and aggregation). These observations suggested that shear stress, like chemical agonists, shows consistent “dose”-and time-response characteristics. Based on these early experiments, it was concluded that the threshold stress for any specific response depends on two factors: (1) platelet-surface interactions and (2) the duration that platelets are subjected to the shear stress. These prescient studies not only led the field of platelet physiology to develop a novel paradigm of stimulus-response coupling within Virchow’s triad, but they formed the basis for the new and fertile field of biorheology.

To investigate the mechanism of the direct effects of shear on platelets it therefore became necessary to attempt to eliminate platelet-surface interactions from the experimental systems. Although platelet reactions with the solid surfaces of damaged blood vessels are essential for hemostasis and thrombosis, in vitro studies of the direct effects of shear stress on platelets (ie, effects independent of platelet-surface interactions) require that surface interactions be minimized or eliminated. Eliminating platelet-surface interactions is important not only because it allows one to investigate the direct effects of bulk fluid shear stress on platelets in vitro, but also because it provides a model in which to investigate mechanisms of thrombosis under shear stress conditions that mimic arterial stenosis in the presence of an intact vascular endothelium.

The instrument of choice for experiments to investigate the importance of bulk shear stress versus surface effects came to be a rotational viscometer. There are many rotational viscometer prototypes, the mechanical designs of which have been described in detail. Some have been modified to apply pulsatile shear stress, and others have been adapted to allow real-time optical measurements of platelet aggregation and calcium flux. The most used designs are (1) a stationary bob placed within a rotating concentric cylinder.
(the Couette viscometer); and (2) a rotating cone whose center is placed in proximity (~25 μm) to the center of a flat plate (the cone-plate viscometer). Both designs generate a constant and uniform shear stress within the liquid suspension. The cone-plate viscometer offers the advantage of an open suspension, allowing for fairly rapid removal of liquid for analyses (Fig 2). Cone-plate viscometers can also be used to analyze platelet-surface reactions by anchoring cells and/or extracellular material on the plate.26-28 The basic geometry of the cone-plate viscometer does not change with the specific device, and experimental results are remarkably consistent using a variety of cone-plate viscometers. The shear rate in this system is nearly constant throughout the volume between the cone and the plate. It is directly proportional to the rotations per minute of the cone, and inversely proportional to the gap angle between the cone and the plate. The gap angle used to generate arterial-level shear forces is typically in the range of 0.30 to 1.00 degrees, and the viscosity (in poise) of a suspension of cells is constant through the range of shear stresses applied experimentally. Thus, a constant shear stress can be applied to a suspension or a monolayer of cells placed into this rotational viscometer by maintaining a constant revolutions-per-minute of the cone on the plate. This type of rotational viscometer is capable of generating shear stresses from less than two dynes/cm² (venous level) to 20 to 30 dynes/cm² (arterial) to greater than 200 dynes/cm² (as occurs in stenosed coronary, peripheral, or cerebral arteries).

To evaluate directly the effects of bulk fluid shear stress on platelets, it is first necessary to eliminate as thoroughly as possible all platelet-surface interactions in the cone-plate viscometer. Platelet-surface interactions can be minimized by coating the shear stress–generating surfaces with silicone or another nonthrombogenic material. This results in platelet responses to shear remaining nearly constant over a large range of surface to volume ratios.14,17,19,21 The silicone-coated cone-plate viscometer therefore provides researchers with a reliable in vitro system to apply shear stress to cell suspensions for the analysis of bulk phase platelet responses. These devices are used to investigate mechanisms of platelet responses to shear stress, as well as to explore the physiologic or pathologic significance of shear-induced platelet activation and aggregation.

MECHANISMS OF SHEAR STRESS–INDUCED PLATELET AGGREGATION

Much of the early literature about the effects of bulk shear stress suggested that shear stress directly activates platelets, although there was little information about mechanisms.13,14,17 Some workers believed that shear stress did not directly cause platelet activation, but rather caused platelet lysis, thereby yielding extracellular concentrations of stored platelet agonists, principally adenosine diphosphate (ADP), which subsequently activated the remaining platelets.29 However, in 1986 Moake et al.,26 applying information derived from other laboratories using flow systems that model platelet-surface interactions,31-34 determined that platelet aggregation in response to pathologically elevated shear stress is not an artifact of cell lysis, but rather depends on the presence of plasma vWF and functional platelet receptor complexes GpIb/IX/V and GpIIb-IIIa. There is indirect evidence that fibrinogen may be the bridging ligand effecting platelet aggregation at shear stresses below ~12 dynes/cm²;35 however, at shear stresses greater than 10 to 12 dynes/cm², platelet secretion and aggregation depend on vWF and platelet GpIb/IX/V and GpIIb-IIIa, and are independent of plasma or platelet fibrinogen.36-38

vWF is a multivalent, multimeric plasma protein that is essential for platelet adhesion to the subendothelium of damaged blood vessels. vWF has binding sites for platelet GpIb or GpIIb-IIIa, and for various subendothelial constituents, including collagen types I, III, and VI. vWF bridging the platelet GpIb/IX/V complex to the subendothelium leads to adhesion in vivo, and vWF bridging GpIb/IX/V on adjacent platelets leads to the cohesive inter-platelet interactions induced by ristocetin in vitro. Under shear stresses in vitro and in vivo, vWF binding to the GpIb/IX/V complex is critically important for platelet adhesion and aggregation.39 In the plasma milieu under static conditions, vWF binding to GpIIb-IIIa is minimal,40 but when high shear stresses are applied to platelets, vWF binds to GpIIb-IIIa as well as to the GpIb/IX/V complex, and this binding contributes substantially to direct shear-induced platelet aggregate formation.40-42 Larger vWF multimers support shear-induced platelet aggregation more effectively than smaller multimers,43 but the minimal vWF valency requirements for shear-induced platelet aggregation have not been established. ADP, whether released from platelets or derived from red blood cells, contributes substantially to shear stress-induced platelet responses.44 When platelets are pretreated with epinephrine before shearing, a synergistic aggregation response is observed.42,45 These results indicate that the effect of shear forces on platelet thrombus formation within the complex in vivo milieu is modified by chemical agonists. This may be one reason why coronary artery occlusion occurs in patients with relatively low-grade stenoses.

Shear-induced, vWF-mediated platelet aggregation requires functional vWF binding sites on both the platelet GpIb/IX/V complex and GpIIb-IIIa.44,45 It does not require ristocetin, other exogenous agents, or chemical modification of vWF. Evidence accumulated to date indicates that the predominant effect of elevated fluid shear stress is on the platelet vWF receptors, rather than on plasma vWF, affecting an ephemeral state of platelet glycoprotein recepitivity for vWF.40 These shear-sensitive platelet membrane components may be a part of the GpIb/IX/V complex.45,46,47 The mechanism by which this occurs is unknown, but it has been hypothesized that the initial interaction between multimeric vWF and platelet GpIb or serves as a nidus for a growing irregular clump of platelets in an evolving aggregate that is subject to increasing liquid shearing forces.48 This growing irregular clump may also serve as a microenvironment that concentrates various platelet agonists, including epinephrine and ADP, resulting in the amplification of an initial shear-related stimulus. The initial shear-induced triggering event has so far been elusive, suggesting that it is a very subtle
effect that is difficult to "trap" for study by extant experimental techniques.

Elevations of platelet cyclic AMP or cyclic GMP inhibit shear-stress-induced platelet adhesion and aggregation.\textsuperscript{46-47} In contrast, inhibition of cyclooxygenase by acetylsalicylic acid has little effect on the initiation of aggregation in response to shear stress\textsuperscript{3}; this may account for the relative lack of potency of aspirin as antithrombogenic therapy for some arteriolar thrombotic disorders. Except for EDTA (see below), most anticoagulants have little effect on shear-stress-induced platelet responses. For example, one study shows that the extent of shear-induced platelet aggregation in a correlate viscometer is similar in PRP anticoagulated with sodium citrate, recombinant hirudin, unfractionated heparin, and low-molecular-weight heparin (LMWH).\textsuperscript{48} However, results from other studies suggest that there may be a small inhibitory effect of unfractionated heparin on shear-induced platelet aggregation\textsuperscript{49} and a modest inhibitory effect of combined hirudin and LMWH on shear-dependent platelet thrombus formation on ex vivo perfused diseased vessels.\textsuperscript{50} Shear stress-induced platelet aggregation is, however, inhibited by fibrinolytic agents.\textsuperscript{51} This effect is caused by plasmin-mediated proteolysis of vWF rather than proteolysis of membrane glycoproteins.

**SHEAR STRESS-INDUCED PLATELET SIGNALING**

The mechanisms by which shear stress induces platelet aggregation are not yet precisely defined, but specific intracellular activation signals are involved in the process. When suspensions of washed platelets are subjected to different levels of uniform shear stress in an optically modified cone and plate viscometer capable of simultaneously monitoring cytoplasmic ionized calcium ([Ca\textsuperscript{2+}]\textsubscript{i}) and aggregation of platelets, a 10-fold increase of [Ca\textsuperscript{2+}]\textsubscript{i} develops. This elevation of [Ca\textsuperscript{2+}]\textsubscript{i}, is accompanied by synchronous aggregation, and both responses are dependent on the shear force and vWF.\textsuperscript{52} EGTA chelation of extracellular Ca\textsuperscript{2+} completely inhibits vWF-mediated increases in [Ca\textsuperscript{2+}]\textsubscript{i}, and aggregation responses to shear stress. Blockade of vWF binding to GpIb\textsubscript{a} also completely inhibits both of these platelet responses to shear stress. The tetrapeptide RGDS and monoclonal antibodies that inhibit the binding of cohesive ligands to GpIIb-IIIa are less effective inhibitors of shear stress-induced increases in [Ca\textsuperscript{2+}]\textsubscript{i}, and platelet aggregation.\textsuperscript{53,54} Blocking the effect of ADP released from stimulated platelets inhibits shear stress-induced platelet aggregation without affecting the shear-induced increase in [Ca\textsuperscript{2+}]\textsubscript{i}.\textsuperscript{55} Neither the [Ca\textsuperscript{2+}]\textsubscript{i} nor aggregation response to shear stress is inhibited by blocking platelet cyclooxygenase with acetylsalicylic acid.\textsuperscript{56}

These results indicate that GpIb\textsubscript{a} and extracellular Ca\textsuperscript{2+} are absolutely required for vWF-mediated [Ca\textsuperscript{2+}]\textsubscript{i}, and aggregation responses to shear stress. Shear stress-induced elevations of platelet [Ca\textsuperscript{2+}]\textsubscript{i}, but not aggregation, are independent of the effects of released ADP, and both responses occur independently of platelet cyclooxygenase. These results are consistent with the model that shear stress induces the initial binding of vWF multimers to platelet GpIb\textsubscript{a}. The vWF-GpIb\textsubscript{a} interaction then causes an increase of [Ca\textsuperscript{2+}]\textsubscript{i}, and platelet aggregation, both of which are potentiated by vWF binding to the activated platelet GpIIb-IIIa complex in the presence of released ADP.

To validate the hypothesis that vWF binding to platelets causes activation resulting in secretion and aggregation, shear stress-induced vWF binding to platelets was investigated. Using complementary approaches (radioligand binding and immunologic tags identified by flow cytometry), direct measurements of shear-induced vWF binding have been obtained.\textsuperscript{56,57,58} vWF binding to platelets under shear has been difficult to quantify because of the variables of vWF multimeric composition, and platelet secretion of stored vWF from α-granules. Nonetheless, certain findings have been consistent: shear stress induces less total vWF binding than does ristocetin, only a minority of platelets bind vWF, the binding is reversible and not saturable, and both GpIb\textsubscript{a} and GpIIb-IIIa contain the sites of shear-induced vWF binding. Binding studies suggest that vWF attachment to GpIb\textsubscript{a} precedes vWF binding to GpIIb-IIIa, a sequence that is consistent with the hypothesis that the initial vWF-GpIb/IX/V interaction activates the GpIIb-IIIa complex.\textsuperscript{59,60} Shear stress-induced activation of GpIIb-IIIa, reported as an increase in monoclonal antibody PAC-1 fluorescence, has been shown.\textsuperscript{54}

Efforts have been made to determine how shear stress-induced vWF binding to GpIb\textsubscript{a} initiates signals for activating the GpIIb-IIIa complex. Although the specific second messengers involved in switching GpIIb-IIIa into a ligandreceptive conformation are not yet identified, the switch involves a network of signals including [Ca\textsuperscript{2+}]\textsubscript{i}, protein kinase C (PKC), and other serine-threonine kinases, tyrosine kinases, and phosphatidylinositol 3-kinases.\textsuperscript{55-59} Within this context, it has been observed that platelet aggregation induced by a pathologic level of arterial wall shear stress (90 dyes/cm\textsuperscript{2}) is associated with the phosphorylation of pleckstrin, an M\textsubscript{r} 47,000 PKC substrate (p47).\textsuperscript{59} Shear-induced p47 phosphorylation depends entirely on vWF binding to platelet receptors, and the inhibition of PKC activity suppresses the aggregation response to shear. Interestingly, in contrast to activation by chemical agonists, shear stress-induced platelet PKC activation occurs independently of any measurable change in diacylglycerol or hydrolysis of phosphatidylinositol 4,5-bisphosphate. These results indicate that when mechanical shear stress induces vWF to bind to platelet GpIb\textsubscript{a}, a diacylglycerol-independent pathway of PKC is activated that contributes to platelet aggregation. The molecules responsible for activating PKC under these conditions are not yet known. Platelet tyrosine kinases are also activated in response to pathologic shear stress (90 dyes/cm\textsuperscript{2}), although the functional importance of this is uncertain.\textsuperscript{60} As with shear stress-induced elevations of [Ca\textsuperscript{2+}]\textsubscript{i}, and PKC, tyrosine kinase activation in response to shear in most cases also depends on vWF binding to platelet GpIb\textsubscript{a} and GpIIb-IIIa. Cytochalasin D, which inhibits GpIb\textsubscript{a}/cytoskeletal interactions, inhibits the tyrosine phosphorylation of an M\textsubscript{r} 76,000 substrate. This M\textsubscript{r} 76,000 protein, along with p66\textsuperscript{SHC}, has been observed to translocate to the platelet cytoskeleton during the course of shear-induced platelet activation, and
this is associated with functionally significant phosphatidylinositol 3-kinase (PI 3-kinase) activity. These results suggest that tyrosine phosphorylation and PI 3-kinase activity may be involved in shear stress–induced aggregation.

Although the molecular mechanisms of shear stress–induced stimulus–response coupling are presently unknown, most signaling is likely to be initiated by vWF binding to platelet GpIbα. GpIb is a transmembranous heterodimeric leucine–rich glycoprotein having M, 165,000 (composed of GpIbα M, 143,000 disulfide–linked to GpIbβ M, 22,000) that forms a noncovalent complex with GpIX and GpV; there are approximately 25,000 GpIbIX/V complexes per platelet. The intracytoplasmic domain of GpIbα interacts extensively with the platelet cytoskeleton. This interaction between the primary receptor for vWF and the submembranous cytoskeleton appears to form a component of the transmembranous structural apparatus linking extracellular shear forces with intracellular signaling. The GpIbα chain can be lengthened by a genetic polymorphism that adds 13 amino acid tandem repeats to the mucin-like macroglycopeptide region, and this had led to speculation that this region may be susceptible to shear-induced conformational alterations.

Once bound to vWF, platelet GpIbα appears to function as a signal molecule, although the mechanism of GpIbα–initiated signaling is not characterized. One hypothesis proposed to explain the mechanism of shear–induced platelet activation states that platelet GpIbα, following shear–induced binding of vWF, undergoes a conformational change that directly, or indirectly through one or more coupling proteins, triggers signals for cellular activation. Studies of thrombin–platelet interactions have shown that α-thrombin binds to platelet GpIbα and that this may affect platelet signal transduction. There is also indirect evidence in support of the hypothesis that the GpIbα/IX/V complex is involved in signal transduction: cAMP, which inhibits platelet activation, promotes the phosphorylation of the β-chain of GpIb and decreases thrombin binding to platelets.

Shear stress–induced vWF–mediated platelet signaling develops generally under conditions associated with aggregation, suggesting that vWF bridging platelets is required for shear stress–induced activation, and that vWF–mediated platelet signaling is inseparable from vWF–mediated aggregation. This phenomenon is different from what happens to platelets activated by strong chemical agonists such as α-thrombin, which stimulate phospholipase C–mediated second messenger formation even in unstimred and unaggregated suspensions.

Studies by Huang and Hellums show that both collision frequency (the number of platelet–platelet contacts per unit time) and collision efficiency (platelet–platelet collisions that result in cohesion, or aggregation) are directly related to platelet concentration and shear stress. Therefore, it is likely that shear stress promotes platelet aggregation, at least in part, by increasing both the number and efficiency of platelet–platelet collisions. Increased collision efficiency at higher shear stresses may result from the direct effects of shear stress on platelets (perhaps specifically on GpIbα/IX/V and GpIIb-IIIa) to induce shear–dependent vWF binding resulting in platelet aggregation. It is also possible that an ephemeral alteration and “activation” of vWF occurs under the influence of pathologic shear stress, although direct evidence in support of this is currently lacking. A primary goal in this field of research is to untangle and rank the various factors in Virchow’s triad that contribute to the initiation of shear stress–induced vWF–mediated platelet aggregation. It is becoming increasingly clear that signal transduction mechanisms that mediate platelet aggregation by chemical stimuli (agonist–receptor interactions) and shear stress may be distinct.

**SHEAR STRESS–INDUCED PLATELET ADHESION**

In humans, physiologic time–averaged mean shear stress levels in the arterial circuit reach 20 to 30 dynes/cm² (shear rates of whole blood equal to 500 to 750 s⁻¹), and pathologic levels (ie, as in a stenosed coronary artery) may reach >350 dynes/cm² (shear rate of 8,750 s⁻¹). The latter clinical situation is often associated with platelet thrombus formation. As described above, pathologic stenosis can directly lead to shear–induced aggregation of platelets from the blood in constricted arteries. Elevated liquid or wall shear stresses also lower the threshold concentration at which platelets become activated in response to chemical agonists, such as epinephrine. Of equal or greater importance, however, is adhesion of blood platelets onto exposed atherosclerotic subendothelium in a region of endothelial cell desquamation (for example, after atherosclerotic plaque rupture), with extensive subsequent platelet aggregation.

There are many established systems for the in vitro investigation of platelet thrombus formation after adhesion, some of which are described briefly in Table 2. These experimental systems have yielded extensive data that form the foundation for many of the conclusions presented in this review. It is interesting to note that, despite the heterogeneity of design in constructing in vitro models of arterial thrombosis, identical bioengineering principles define the majority of the phenomena observed in different flow chambers. Consequently, some general conclusions can be made regarding mechanisms of arterial thrombosis initiated by platelets adhering to damaged blood vessels in vivo.

To begin, one should first describe the phenomena observed in any flow chamber into which “subendothelium” is placed. However, the interpretation of such phenomena must account for differences in the composition of the subendothelial tissue depending upon the site and species from which it is derived. Nonetheless, it is clear that in all cases platelets adhere, change shape (“spread”), and aggregate (“thrombus formation”) when whole blood is perfused over de–endothelialized mammalian vessel segments. These responses depend on mechanical shear forces. The range of shear stresses over which platelet adhesion and subsequent aggregation are observed is approximately 1 to 200 dynes/cm². Within the range of elevated shear stresses (>30 dynes/cm²), platelet thrombus production depends on vWF binding to platelet GpIbα/IX/V and GpIIb-IIIa. The source of vWF can be plasma or platelet α-granules or the subendothelial extracellular matrix. Larger vWF...
multimers are more effective than smaller vWF multimers at promoting platelet thrombus formation in perfusion experiments. Platelet GpIb/IX is involved in the initiation of adhesive platelet-subendothelial interactions under arterial level shear stress, and vWF attachment to GpIIb-IIIa is required for subsequent aggregation and thrombus propagation. Acetylsalicylic acid treatment of blood does not inhibit shear-dependent platelet thrombus formation in parallel plate flow chambers, but it has been reported to have a moderate inhibitory effect in other flow systems. PG\textsubscript{II}a (prostacyclin) derived from endothelial cells inhibits shear-induced platelet thrombus formation on subendothelium.

Shear-induced platelet thrombus formation appears to promote the generation of fibrin through the activity of soluble coagulation factors. This is very important because, in the arterial circulation, the higher shear stresses associated with increased flow tend to dilute out certain coagulant molecules, such as fibrinogen and perhaps prothrombin and thrombin, thereby preventing the formation of insoluble fibrin. The platelet-dependent arterial thrombus represents a dynamic rheological process, however, and as luminal diameter decreases, so does blood flow and flow velocity, such that fibrin generation ultimately develops. Based on in vitro studies of whole blood perfused over vessel wall constituents (eg, collagen) it is clear that the activation of the soluble coagulation systems is a secondary, but significant, event in shear-induced thrombus formation that occurs in vivo after platelet adhesion and aggregation.

Platelets subjected to elevated shear stresses do not adhere to an intact endothelial cell monolayer. Platelets do, however, bind tightly to exposed subendothelium, become activated to express a functional GpIIb-IIIa receptor complex, and subsequently aggregate. Many candidate proteins have been studied in perfusion systems in attempts to define the critical vessel wall factors operating in Virchow’s triad of arterial thrombosis. Collagen, insoluble vWF, fibrinogen, thrombospondin, laminin, and fibronectin have been investigated as potential mediators of shear-induced platelet-surface interactions. In addition, platelets interact with prosthetic surfaces under shear conditions, and these interactions mediate vascular graft occlusions.

Collagen. In constructing a model of subendothelium, the following observations are relevant: (1) fibrillar collagen types I and III are present in highest concentrations, along with collagen types IV, V, and VI, in human adult arteries; (2) most of the collagen in the relatively pristine subendothelium of arterial vessels during childhood is type III; (3) both collagen types I and III increase in the thickened subendothelium of atherosclerotic arteries; however, most of the accumulating collagen is type I; and (4) collagen types I, III, and VI bind to vWF. Monomeric vWF contains two binding sites for collagen types I and III, only one of which may be physiologically relevant. The vWF binding site for type VI collagen is uncertain.

Most studies of shear-induced platelet-collagen interactions in the three types of perfusion chambers have used morphometric or radioactive techniques to evaluate platelet accumulation at the end of an experiment. Recently, the combination of parallel plate perfusion (Fig 3) with computerized epifluorescence videomicroscopy has allowed the observation, videotaping, and three-dimensional representation of platelet adhesion and aggregation in real-time. Because results have been generally consistent using different systems, a description of the dynamics of platelet-collagen interactions in the parallel plate flow chamber in real-time will be used as a paradigm of the basic mechanisms of platelet thrombus formation over bovine type I collagen monolayers in the presence of shear stress. It should be noted that there is experimental evidence using an annular perfusion chamber that collagen is not the primary vWF binding site within a complex subendothelial milieu. However, more recent studies of transverse sections of human coronary artery atheromatous plaques show that the “thrombogenicity” of excised endothelialized plaques subjected to 64 dynes/cm\textsuperscript{2} shear stress in a parallel plate perfusion chamber results only from plaques containing types I and III binding to platelets through a process that depends, at least in part, on plasma vWF.

In the parallel plate flow chamber, the number of individual platelets and platelet monolayers per unit area, a measure of platelet adhesion, increase during the initial 15-second exposure to 60 dynes/cm\textsuperscript{2} wall shear stress. As individual platelet thrombi develop and merge into larger aggregates at 30 and 60 seconds of flow, the number of individual adhesive events decrease concomitantly. In this system, the total number of platelets deposited from normal blood onto
collagen-coated slides using different anticoagulants (0.38% citrate, 10 U/mL unfractionated porcine heparin, 1 U/mL LMWH, or 200 U/mL recombinant hirudin) is not significantly different.

Three-dimensional reconstructions of the thrombi formed using blood from a patient with severe vWD in the presence or absence of purified vWF are shown in Fig. 4. In severe vWD blood (with <1.5 U/dL plasma vWF and undetectable platelet vWF forms) without vWF, platelet adhesion after 60 seconds’ exposure to 60 dynes/cm² is much less than that observed using severe vWD blood in the presence of 100 U/dL of purified vWF. Because of the decreased adhesion in severe vWD blood, subsequent platelet aggregation is also considerably diminished. This is further evidence in support of the conclusion that there is direct adhesion-aggregation coupling. Figure 4 also shows that the release of stored vWF from shear-activated platelets has a significant effect on platelet thrombus formation. Comparative studies of severe vWD platelets in normal plasma, versus normal platelets in severe vWD plasma, confirm that both plasma vWF and vWF released from platelets contribute to platelet thrombus formation. Platelet adhesion onto collagen-coated surfaces is inhibited by a monoclonal antibody to GpIba. Under identical shear conditions, there is greater platelet adhesion onto collagen using severe vWD blood than in the presence of an anti-GpIba antibody (~30% v ~5% of controls, respectively), suggesting that only a small quantity of vWF immobilized with collagen is sufficient to initiate platelet adhesion via GpIba. Either an anti-GPllb-IIIa antibody, or an antibody against the RGD sequence in vWF that mediates vWF binding to GPllb-IIIa, is less effective as an inhibitor of platelet surface accumulation than the anti-GpIba antibody. In platelets deficient in GPllb-IIIa, or in the presence of inhibitors of GPllb-IIIa, platelet adhesion is minimally inhibited. In contrast, subsequent platelet aggregation is almost eliminated. ADP promotes platelet aggregation subsequent to initial adhesion, but blockade of platelet cyclooxygenase has no significant effect on either platelet adhesion or aggregation under the conditions of these perfusion experiments.

It is uncertain if direct collagen-platelet interactions potentiate vWF-dependent platelet adhesion onto insolubilized collagen fibrils. Under nonshear conditions platelets directly bind collagen through at least three receptors (GpIla/IId, CDIV, and CDVI). GpIla/IId-initiated platelet-collagen interactions under low shear conditions operate through the activation of the GPllb-IIIa complex.

vWF. Compared with vWF multimers from normal plasma, insoluble subendothelial vWF derived from endothelial cells may be more active in initiating platelet adhesion under flow. Insoluble vWF alone does not support rapid platelet attachment in the absence of shear forces. However, the threshold wall shear stress for platelet adhesion to vWF-coated surfaces appears to be somewhat lower than the threshold liquid shear stress for direct platelet aggregation mediated by soluble vWF. Both GpIla and GPllb-IIIa are involved in shear-dependent platelet adhesion onto vWF-coated perfusion chambers.

It is known that vWF becomes rapidly insolubilized onto the exposed subendothelium of human arteries, as well as onto collagenous and noncollagenous components of the vessel wall, and that these events may precede and augment platelet adhesion. As previously described, in vitro experiments indicate that vWF multimers immobilized with human collagen type I attach predominantly to platelet GPllb/IX/V receptors, suggesting that a single platelet site for vWF binding is exposed under these conditions to effect adhesion. The relative importance of direct platelet-collagen binding compared to direct platelet-vWF binding within a complex subendothelial milieu is not established, although studies from excised atheromatous human coronary arteries provide data that both are involved in platelet adhesion under elevated shear stress conditions.

Fibrinogen. Plasma fibrinogen is cleaved by thrombin to fibrin monomers that insolubilize by polymerization and are subsequently cross-linked by a transglutaminase (factor XIII). Fibrinogen can be found on the surface of vascular endothelium, and fibrinogen and fibrin are present in atherosclerotic plaques. Fibrinogen is also deposited on vascular prostheses, where it is the major ligand mediating platelet thrombus growth on artificial surfaces. Platelets deposit onto fibrin-coated coverslips in a perfusion chamber at 12, 18, and 52 dynes/cm². At the highest of these shear stresses platelet thrombus formation on fibrin is relatively more dependent on vWF and GpIla, whereas at the lower shear stresses platelet thrombus formation on fibrin is relatively more dependent on GPllb-IIIa. It has been suggested that under pathologically elevated shear stress conditions, GPllb-IIIa binding directly to fibrin "slows down" the passing platelets, but that vWF bridging fibrin...
Platelets and shear

Severe vWD Blood, 60 sec of Flow

Severe vWD Blood + 100% Purified vWF
60 sec of Flow

Healthy Donor, 60 sec of Flow

Fig 4. These are computer-generated three-dimensional topographic representations of platelet thrombi formed on Bovine type I collagen. The results presented show adhesion (x and y planar surface) and aggregation (z-axis height) of platelets from citrate-anticoagulated whole blood subjected to 60 dynes/cm² for 60 seconds. Note that replacing plasma vWF levels to normal only partially restores the adhesion and aggregation of platelets on type I collagen. This is so because platelet secretion of stored vWF is required for optimal adhesion and aggregation in response to elevated shear stress. (Reprinted with permission.)

Platelet adhesion to GpIbα "locks" the platelets into a stable thrombus capable of remaining attached under high shear stresses. The vWF binding site(s) on fibrin(ogen) have not been identified.

Other subendothelial matrix proteins. Platelet adhesion to fibronectin occurs only at low shear stresses (eg, 0 to 12 dynes/cm²). Evidence has been presented that this adhesion is partly dependent on the platelet integrins GpIIb-IIIa and GpIc/IIa (VLA 5), which directly interact with fibronectin. Adhesion is also completely dependent on vWF and GpIbα. Because there is no evidence that either vWF or GpIb/IX/V binds directly to fibronectin, these results suggest that adhesion is maintained only when vWF forms an adluminal interplatelet scaffolding through GpIb/IX/V connections. Fibronectin may also enhance platelet accumulation on collagens type I and III under low shear conditions. Lammin also supports platelet adhesion under low shear conditions (4 dynes/cm²). This depends on platelet VLA-6, but is independent of vWF, GpIIb/IX/V, and GpIIb-IIIa. Thrombospondin supports platelet adhesion in a shear stress-dependent manner, with maximal adhesion at 64 dynes/cm². This adhesion is also apparently independent of vWF, GpIb/IX/V, and GpIIb-IIIa. The platelet receptor that binds thrombospondin under shear conditions in these studies has not been identified, but is not GpIIb-IIIa, GpIa/IIa, GpIV, or αvβ3.

Effects of shear stress on vessel wall regulation of platelet thrombus formation

Situated at the interface between blood and the vessel wall throughout the circulatory system, endothelial cells are strategically positioned to respond to hemodynamic forces. These forces include shear stress and strain. Strain is the abluminal force that results from wall shear (the force which would "tear" a cell from its adherent state), resulting in
vascular distention that affects endothelial cells and subendothelial components (e.g., smooth muscle cells and extracellular matrix). Flow-induced hemodynamic forces signal endothelial cells to modulate the synthesis and release of a variety of vasoactive and antithrombotic substances, the general net effect of which is to promote blood fluidity. The phenomenon of flow-induced adaptive vasodilation is mediated by the release of vasoactive products, such as PGI$_2$ (prostacyclin) and endothelium-derived relaxing factor or nitric oxide (NO), from shear-stimulated endothelial cells.

Endothelial cells may act as shear sensors in their role of regulating flow-dependent arterial diameter under normal and pathologic conditions. In addition, shear stress induces endothelial cells to release platelet inhibitory and fibrinolytic products, which, in the presence of intact endothelium, tend to counterbalance the proaggregatory effects of pathologically elevated shear stress in the arterial circulation.

PGI$_2$ acts as both a potent vasodilator and inhibitor of platelet aggregation by raising intracellular levels of cAMP in vascular smooth muscle cells and platelets, respectively. Shear stress stimulates PGI$_2$ synthesis by endothelial cells.

Using a parallel-plate flow chamber with an immobilized monolayer of endothelial cells, it has been observed that the sudden onset of flow abruptly increases PGI$_2$ production, followed by a decrease in production to a steady state within several minutes. The steady-state release of prostacyclin by endothelial cells subjected to pulsatile shear stress is more than double that released by cells exposed to constant shear stress.

These findings indicate that endothelial cells release bursts of PGI$_2$ in response to step increases in shear stress. PGI$_2$ is likewise produced by endothelial cells subjected to the cyclic strain or stretch which occurs with each pulse wave.

NO is also both a potent vasodilator and an inhibitor of platelet adhesion and aggregation. These effects are mediated by elevations of intracellular levels of cyclic GMP in smooth muscle cells and platelets. NO is the by-product of the conversion of L-arginine to L-citrulline by a family of NO synthases (NOS).

One of the isoforms of this enzyme is a membrane-associated, Ca$^{2+}$/calmodulin-dependent, constitutive NOS (cNOS), which is found in endothelial cells (hence termed "eNOS") and can be distinguished from the constitutive NOS present in neuronal tissue. The release of NO from endothelial cells is stimulated by both shear stress and cyclic strain. The stimulation of endothelial NO production in response to both shear stress and cyclic strain is greater when flow is pulsatile.

Similar to the observations with shear-induced PGI$_2$ production, exposure of endothelial cells to the sudden onset of laminar flow results in a rapid burst of NO release that increases further with the superimposition of increases in flow above the preexisting level. Because eNOS is Ca$^{2+}$/calmodulin-dependent, the initial rapid endothelial cell synthesis of NO in response to shear stress (as well as chemical agonists) has an obligatory requirement for an increase in the concentration of cytosolic free Ca$^{2+}$.

Tissue plasminogen activator (tPA)-generated plasmin is not only fibrinolytic but can also proteolyze large vWF multimers and, under certain conditions, inhibit platelet function. As noted previously, tPA and plasmin inhibit shear-induced platelet aggregation. Arterial levels of shear stress (>15 dynes/cm$^2$) stimulate secretion of tPA by endothelial cells, whereas the secretion of plasminogen activator inhibitor type 1 (PAI-1) remains unaffected by shear stress over the physiologic range. This enhancement of the fibrinolytic potential of endothelial cells in response to hemodynamic forces is at least partly transcriptionally regulated, as tPA mRNA is increased by laminar shear stress.

The mechanisms by which the principal hemodynamic forces of shear stress and cyclic strain transduce signals to alter the structural and functional properties of endothelial (and other) cells are poorly understood. In addition, fluid shear stress and cyclic strain may use different signaling pathways, because downstream gene regulation and protein secretion are quite different in response to these two mechanical stimuli. "Mechanotransduction" may be initiated directly, by shear-induced changes in the state of flow sensors at the luminal surface of endothelium or elsewhere in the cell after transmission of the force via the cytoskeleton. Alternatively, mechanotransduction may occur indirectly by changes in local endothelial cell surface concentrations of chemical agonists created by the convective transport of these mediators from the bulk fluid to the cell boundary layer. As reviewed by Davies and Tripathi, hemodynamic forces generate a diverse set of intracellular signals in endothelial cells: some signals develop extremely rapidly, and these may be followed after several hours by altered gene expression and by cytoskeletal reorganization if the forces are sustained. Rapid responses to flow may involve second messengers that are indistinguishable from those that result from chemical agonist-receptor coupling in vascular cells, including activation of different ion channels, increases in cytosolic free Ca$^{2+}$ and membrane phosphoinositide turnover with the formation of inositol 1,4,5-trisphosphate (IP$_3$) and activation of PKC.

It has been proposed that rapid responses to flow may activate two signal transduction pathways in endothelial cells. One pathway is Ca$^{2+}$-dependent and involves activation of phospholipase C and increases of [Ca$^{2+}$], whereas a second pathway is Ca$^{2+}$-independent and involves a guanine nucleotide regulatory (G) protein-mediated activation of PKC and mitogen activated protein (MAP) kinases. In contrast to these second messenger-mediated rapid responses, a delayed response of endothelial cells to flow may reflect the upregulation of expression of certain genes. The pathways through which externally applied hemodynamic forces signal the endothelial cell nucleus to activate gene transcription may involve second messengers or cytoskeletal reorganization. In addition, a cis-acting shear stress-responsive element has been identified in the promoter of the PDGF-B gene. This sequence is also present in the promoters of other endothelial cell genes upregulated by shear stress, including the tPA gene. The relationship between shear-stimulated transcription factors and their binding
sites in the promoter regions of shear-responsive genes is an area of considerable interest. The transcriptional factor NF-kB, which accumulates in the nuclei of endothelial cells exposed to fluid shear stress, binds to the shear stress response element and has been implicated in the transactivation of endothelial cell genes responding to rheologic stimuli.\textsuperscript{171}

Genes that do not contain the shear stress-responsive element in their 5' promoter regions, such as the tissue factor gene, also respond to shear stress. More recently, it has been found that the phorbol ester TPA-responsive element (TRE) may be a functional shear responsive element, leading to shear-induced immediate (or early) gene expression in endothelial and other cells.\textsuperscript{172} Further characterization of these and other positive (and negative) shear-responsive regulatory elements, and the proteins that regulate them, will facilitate our understanding of biomechanical stimulation of endothelial cell function.\textsuperscript{173} Thus, observations to date indicate that shear stress stimulates the production of certain endothelial-derived mediators (eg, prostacyclin) by activating constitutive stimulus-response effector mechanisms, and the production of other mediators (eg, tPA) by activating gene transcription.

The modulation of platelet activation by shear-activated vascular cells is more complex than the above-described shear effects on the release of individual endothelium-derived vasoactive and platelet inhibitory mediators. Shear-induced release of endothelium-derived prostaglandins, NO and tPA, may act synergistically to inhibit platelet activation.\textsuperscript{174-176} On the other hand, the platelet inhibitory actions of NO are quenched by hemoglobin in whole blood.\textsuperscript{177} Nevertheless, NO may still be able to inhibit platelet thrombus formation over areas of intact endothelium because, in flowing blood, red blood cells tend to stream to the center of the vessel lumen whereas platelets distribute to the lumen periphery where they could be exposed to inhibitory concentrations of NO. Furthermore, endothelial cells interact, directly or via humoral mediators, with other constituents of the vessel wall (eg, vascular smooth muscle cells and extracellular matrix), which may further modify platelet responses to shear stress.\textsuperscript{125}

In summary, shear stress can directly activate platelets. Under normal circumstances, however, intact endothelial cells exposed to the same rheologic forces produce a potent array of vasodilatory and platelet inhibitory mediators that mitigate the activation of platelets by shear stress to preserve blood fluidity. However, when the vessel wall component of Virchow's triad is perturbed these endothelium-derived factors cannot oppose direct shear stress-induced platelet aggregation and the shear-induced platelet activation and aggregation that occur subsequent to initial platelet-subendothelial adhesion. The result is platelet thrombus formation associated with downstream tissue ischemia and infarction.

HEMOSTASIS

Experimental data from in vitro flow systems and in vivo models of arterial thrombosis provide a solid foundation for the concept that mechanical shear forces affect platelet thrombus formation under conditions of physiologic and pathologic arterial flow. There are also data that provide a reasonable hypothesis to explain why proaggregatory shear stresses under physiological arterial flow (in the vast "microvasculature") don't result in spontaneous thrombosis: shear in the arteriolar circuit simultaneously stimulates endothelial cell release of antiaggregatory factors\textsuperscript{178} (see above). However, these facts do not necessarily explain primary hemostasis. It is established that platelet-vessel wall interactions are required for hemostasis, and that hemostasis depends on vWF, platelet GpIb/IX/V, and platelet GpIIb-IIIa. Primary hemostasis must also occur in the low shear vasculature: venules and capillaries. Is it possible, therefore, to relate shear-dependent mechanisms of thrombosis to mechanisms of hemostasis? Once again, clues that provide a solution to this puzzle are gathered if one places its pieces within the context of Virchow's triad.

If the blood factors responsible for primary hemostasis and thrombosis are held constant, but the rheologic factors that affect hemostasis and thrombosis are different, one might look closely at vessel wall factors that direct a hemostatic response as possibly being different from those that effect thrombosis. As described above, both fibronectin and laminin support low shear-induced platelet adhesion, but the adhesion is vWF-independent. Therefore, it becomes necessary to consider other components of the vessel wall that could be involved in vWF-dependent hemostasis at low shear stresses.

Type VI collagen has been identified as a component of subendothelial microfibrils capable of binding vWF.\textsuperscript{91,179} Type VI collagen is codistributed with vWF in the subendothelium of human umbilical vein endothelial cells in the apparent absence of collagen types I and III. The pattern of distribution of collagen VI in the subendothelium of normal arteries and veins, or atherosclerotic arteries, is not yet known. It has recently been reported that platelet adhesion and subsequent aggregation on purified type VI collagen in a parallel plate flow chamber are extensive at low shear rates (4 dynes/cm\textsuperscript{2}). In contrast, little adhesion occurs at high shear rates (40 dynes/cm\textsuperscript{2}). These shear-related effects are exactly opposite to those observed with collagen type I.\textsuperscript{97} The low-shear platelet deposition on type VI collagen depends on vWF, GpIbα, and GpIbb-IIIa. Experiments using whole blood from a patient with severe vWD suggest that there may also be some vWF-independent interactions between platelets and type VI collagen.\textsuperscript{180} These interactions may be mediated by platelet GpIa-IIa.\textsuperscript{181} It is also possible that platelet adhesion to fibronectin and/or laminin could contribute to vWF-independent adhesion at low shear stresses.\textsuperscript{112-114} Therefore, it is possible that a multistep process develops rapidly in the low-shear environment of cutaneous and mucosal terminal venules and capillaries (eg, in bleeding time wounds) that involves direct platelet interactions with fibronectin, laminin, and/or type VI collagen, and indirect interaction between platelets and type VI collagen through vWF bridges to GpIb/IX/V or GpIbb-IIIa.

CLINICAL CONDITIONS

The importance of shear stress–induced platelet aggregation in human pathology is mostly inferred. The most rele-
vant clinical condition epidemiologically is arterial stenosis caused by chronic atherothrombotic occlusion of the coronary, carotid, and peripheral arteries. There is evidence that agents that interfere with shear stress–induced platelet responses may beneficially affect these diseases and improve clinical outcome.

The in vivo administration of the mouse-human chimeric monoclonal Fab fragment against GpIIb-IIIa (c7E3; 0.25 mg/kg; Centocor, Malvern, PA) to patients in association with coronary angioplasty results in almost complete inhibition of shear-dependent aggregation subsequent to vWF-mediated adhesion onto type collagen I for at least 24 hours after 7E3 infusion.182 These in vitro observations are associated with improved reperfusion and diminished reocclusion of the obstructed coronary artery following angioplasty.183 Integrelin (Cor Therapeutics, South San Francisco, CA), a cyclic KGD-containing heptapeptide that antagonizes the binding of fibrinogen and vWF to platelet GpIIb-IIIa, has been investigated in angioplasty patients also receiving aspirin and heparin. Blood was collected before and during Integrelin infusion (bolus of 135 mg/kg, followed by 0.5 or 0.75 mg/kg min). As with c7E3, vWF-mediated platelet adhesion to collagen I was not inhibited at any time point by in vivo Integrelin. In contrast, using blood collected after 45 minutes of Integrelin infusion, there was a significant decrease in the size of the platelet aggregates that formed subsequent to vWF-mediated adhesion at high shear stress. There was also an associated ~50% inhibition by the infused Integrelin of ex vivo platelet aggregation induced by >100 dynes/cm² shear stress in the cone-and-plate viscometer. No studies to date elucidate the clinical importance of inhibiting vWF-platelet GpIIb/IIIa interactions.

It has been observed that larger vWF forms are considerably more effective, compared with smaller vWF multimers purified from normal cryoprecipitate, in mediating fluid shear stress–induced platelet aggregation (see above). This observation was recently extended to a clinical situation. It has been found that children with chronic relapsing thrombotic thrombocytopenic purpura have "unusually large" (UL) vWF forms in their plasma in association with excessive shear stress–induced platelet aggregation in vitro at 90 to 180 dynes/cm².184 ULvWF forms (normally secreted abuminally by endothelial cells) may be especially effective in promoting the adhesion of platelet GpIIb/IIIa to subendothelium containing collagens I, III, or VI. The excessive release of ULvWF multimers into the bloodstream in response to endothelial cell toxins may be fundamental to the pathophysiology of TTP and the hemolytic-uremic syndrome.185

**FUTURE DIRECTIONS**

It will be necessary to refine further in vitro model systems of shear stress that more closely simulate normal and pathological conditions of flow in vivo. This must be done to elucidate fully the complex interactions between cellular and extracellular matrix components of the vessel wall that are likely to modulate both vascular responses to shear stress, as well as the intravascular responses of platelets to shear stress. The contributions of other blood constituents, particularly red blood cells and leukocytes, have likewise been incompletely defined in the process of platelet-vessel wall interactions under various conditions of shear stress. The observation that elevated shear stress activates platelets via GpIIbα pathways that are different from chemical agonists suggests the possibility of "lesion-specific" anti-thrombotics that affect platelets under conditions of high shear without perturbing the molecular interactions that result in hemostasis. Further investigations are required to determine if this hypothesis is valid.

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