A catch bond is formed that tightens as the platelet is pulled by the flowing blood. This bond triggers microvascular hemostasis and acute atherothrombosis. It is capable of withstanding vessel wall shearing forces that sweep away all other biochemical reactions, such as those leading to leukocyte attachment and fibrin deposition. The bond slips, slow platelets roll or flip as new bonds form, catch, and slip; and stable attachment finally develops through platelet GpVI binding collagen and activated αIIbβ3 binding VWF and fibrinogen. Shear-induced binding of VWF to GpIbα is sufficient for αIIbβ3 activation, and it is noteworthy that Kasirer-Friede and colleagues previously reported that this response is modulated by adhesion and degranulation promoting adapter protein (ADAP) under flow conditions found in arterioles and stenotic arteries (a shear rate of 1500/sec).2

Mechanotransduction is the cellular process by which physical forces are sensed and converted into biochemical signals.3 Molecular mechanisms of mechanotransduction are poorly understood but perhaps best worked out for integrins. Integrins transduce forces only after they are ligand-bound.4 Integrin-mediated mechanotransduction triggers cellular responses aimed at balancing the force applied, whether the force is shear, strain, or stretch.4 In this regard, the integrin is used by a cell to permit compliance with Newton’s third law that “for every action, there is an equal and opposite reaction.” Forces applied to cells bound to matricellular proteins at focal contacts or focal adhesions are transduced from the integrin’s extracellular ligand-binding domain to its β chain’s cytoplasmic domain connected to the cytoskeleton. Integrin-transduced forces cause cytoskeletal deformations and actomyosin-driven cytoskeletal remodeling that generate an opposing force, which in a platelet is the force used to maintain adhesion in the face of flowing blood. The bio-chemical mechanisms by which these responses occur are vague but are thought to be allosteric, reversible, and bidirectional. It is also hypothesized that mechanical forces accelerate chemical reactions, spatial arrangements, and colocalizations that are diffusion-limited under static conditions.5

The article by Kasirer-Friede et al identifies several of the allosteric interactions that operate to enhance mouse platelet adhesion under shear conditions.6 The hypothesis that ADAP regulates the actin cytoskeleton under hydrodynamic forces focuses on integrin-mediated mechanotransduction because of previous work showing that shear stress modulates human αIIbβ3-mediated adherence through a mechanism that involves β3/cytoskeletal interactions and SLP-76/ADAP colocalization;7 that integrin-induced outside-in signaling in static T cells utilizes the ADAP partner SLP-76;8 and that ADAP, because it mediates “inside-out” signaling to αIIbβ3,2 is likely to operate allosterically and therefore bidirectionally.3 By using normal and ADAP deleted mice to examine in vivo carotid thrombosis and ex vivo platelet deposition onto fibrinogen in a flow chamber perfused with blood at a shear rate of 500/sec (recapitulating flow conditions in large arteries and arterioles), these investigators prove that ADAP fine-tunes αIIbβ3-mediated adhesion. ADAP is a component of a cytoskeletal and kinase-mediated signaling cascade that stimulates increased surface contact points through the generation of lamellipodia, a mechanism as teleologically sound as protecting a tent from a windstorm by adding support cables while spreading, flattening, and tethering the tent to the ground with hundreds of additional spikes.

What is the clinical significance of these results? At this time probably distant, as ADAP functions under both physiologic and pathologic shear conditions, suggesting that an ADAP inhibitor would have antithrombotic effects separate from any antithrombotic activity. In addition, because ADAP is a protein found in all hematopoietic cell lineages, its inhibition would carry the risk of immune suppression, as demonstrated by children who suffer severe bleeding and recurrent infections from a point mutation in kindlin-3 that disrupts its integrin-activating function.9 Nonetheless, it is my view that this study is more important than its translational potential because it excites the process of blending clinical medicine with mechanical engineering (i.e., bioengineering) within our community of hematologists. Virchow’s conceptual triad of the pathogenesis of thrombosis (perturbations in blood, blood vessel, and/or blood flow) has done far better than simply withstanding the test of time. It has provided a conceptual framework for discoveries inconceivable 150 years ago. Considering that Virchow identified “stasis” as a cause of thrombosis, he probably would have scoffed at the idea that “fluidity” can also cause thrombosis. But today, after more than a century of technological developments represented by work like that of Kasirer-Friede et al, one can imagine that Professor Virchow, if given the chance, would be delighted—and enlightened enough—to aggressively seek a collaboration with Sir Isaac Newton.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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THROMBOSIS & HEMOSTASIS

Comment on Rand et al, page 2292

A novel antiphospholipid antibody agent?

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In this issue of Blood, Rand and colleagues have shown that in vitro hydroxychloroquine can reverse antiphospholipid antibody-mediated disruption of the annexin V A crystal lattice that protects phospholipid bilayers from taking part in coagulation reactions. The protective effect of hydroxychloroquine against antiphospholipid antibody-induced damage was seen on both cultured endothelial cells and a syncytialized trophoblast cell line.
Since the discovery of antiphospholipid antibodies (aPLs) and their associated thrombotic and pregnancy complications nearly 30 years ago, we have learned an enormous amount about the clinical manifestations of antiphospholipid syndrome, while animal and in vitro work has developed possible models of pathogenesis. However, there have been no major advances in treatment. We remain heavily reliant on anticoagulation in preventing both aPL-related thromboses and pregnancy morbidity. Both patients and physicians alike struggle with the stresses and strains of managing anticoagulant monitoring and the risks of major bleeding, especially with the oral vitamin K antagonists. The current hope is that the new oral fixed-dose anticoagulants will bring better efficacy, safety, and ease of use.

Current models of how aPLs cause damage are sophisticated. But, it can be argued that up to now, no single pathogenic model can both have the potential to be applicable to all the heterogeneous types of aPL and explain the primary moments of how aPL produces changes that activate coagulation. This lack of understanding of how aPLs cause damage prevents the development of novel agents to tackle the antibody head on. Rand et al’s hypothesis that the primary damaging event of an aPL is to disrupt the annexin A5 shield is a plausible mechanism to explain all the phenotypes of antiphospholipid syndrome, for the shield is present on the cell types (such as endothelium, platelet, and trophoblast) affected by aPL. If this hypothesis holds true, then hydroxychloroquine is possibly the first treatment targeted at the mechanism of action of the antibody, that is, a treatment that could “block” the primary effects of the antibody, rather than our existing crude approach of using anticoagulation to switch off the downstream effects of aPLs.

Hydroxychloroquine has been available since the 1950s. It is traditionally known as an antimalarial agent, but also a well-established anti-inflammatory agent used as a first-line treatment for the fatigue, arthralgia, and skin rashes of systemic lupus erythematosus. It has an established safety profile including reassuring data on its use during pregnancy, an important consideration in view of the pregnancy morbidity associated with antiphospholipid syndrome.

Hydroxychloroquine has been known to have immunomodulatory effects for a long time. Recent work has shown this is partly or wholly mediated by its effects on inhibiting Toll-like receptors on dendritic cells. Interestingly, over the past 10 years, cohort studies of lupus patients have identified that those patients receiving hydroxychloroquine, whether they have antiphospholipid antibodies or not, have a significantly lower risk of thromboses. Another clue comes from a mouse model of APS in which mice receiving hydroxychloroquine had smaller and shorter-lasting thrombi after injury than those receiving placebo. Hydroxychloroquine has been known to mildly inhibit platelet aggregation for a long time, but the effect was not thought to explain its clinical antithrombotics efficacy. So, Rand et al’s description of its direct effects against aPL is an interesting alternative.

Of course, no paper is without shortcomings and as attractive as the theory may be, further evidence is required before we can be sure that this is not a false dawn. The described studies, elegant as they are, used aPL from only 3 patients, and the work needs repetition with other aPL. Does current treatment with hydroxychloroquine attain levels similar to those used in these in vitro studies? Does hydroxychloroquine attain the same effects in patients with aPL without systemic lupus erythematosus?

Certainly, however, the combined evidence from the cohort studies of hydroxychloroquine effect in lupus patients, Rand’s work, and a favorable safety profile suggest trials of hydroxychloroquine in the primary and secondary prevention of thrombosis and pregnancy morbidity of aPL are timely. The practical challenge to the community of aPL physicians is to prevent hydroxychloroquine usage slipping into routine clinical practice without first organizing good multicenter clinical trials to assess its true utility.

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