genes, is an important finding with clear implications for understanding the pathogenesis of (and potentially developing more effective targeted therapy for) this high-risk group of AMLs. It is also clear that the concept of a role for aberrant DNA methylation and the components of the epigenetic machinery in the pathogenesis of AML continues to gain momentum, and we can expect to see more examples of these relationships emerging in the near future.

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

REFERENCES

**PLATELETS & THROMBOPOIESIS**

Comment on Yong et al, page 11

**An event of shear importance**

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In this issue of *Blood*, Yong and colleagues report that circulating blood platelets and monocytes in stable coronary artery disease patients can become activated by shear stress during passage through a stenosed sclerotic lesion, despite dual antiplatelet therapy, and the absence of either plaque rupture or collagen exposure.1

A remarkable feature of the Yong et al study was the collection of blood samples from the coronary artery both upstream and downstream of the coronary lesion, as well as from the coronary sinus. These multiple samples were combined with the application of new computational angiographic imaging technology so that cellular/molecular changes in platelets or monocytes could be correlated with the severity of stenosis and the calculated degree of intravascular shear stress. Blood platelets from 20 patients with stable angina undergoing elective percutaneous coronary interventions involving an epicardial coronary artery showed significantly elevated surface expression of the α-granular activation marker, P-selectin, and increased platelet-monocyte aggregates downstream of the stenosis.1 There was no significant increase in the platelet levels of activated integrin αβ3 which binds von Willebrand factor (VWF) or fibrinogen in platelet aggregation, possibly because all the patients studied were on the antiplatelet drugs, aspirin and clopidogrel (Plavix). The data suggest that levels of shear stress under these conditions are sufficient to activate platelets leading to degranulation and monocyte interaction, but without shear–induced platelet aggregation, raising important questions regarding the efficacy of antiplatelet drugs in cardiovascular disease.

Activation of blood platelets by rheologic shear stress has been known for decades, stemming from studies of artificial heart
Shear-induced activation of vascular cells in vitro and in vivo. (A) Diagram of the cone-plate viscometer, where a rotating cone on a fixed plate exerts uniform shear stress on a sample of blood or platelets without exposure to a thrombogenic surface. (B) Shear stress–exposed blood passed through a stenotic coronary artery shows signs of activated platelets (increased P-selectin expression) and activated monocytes (increased CD11b) when blood samples (vertical arrows) are taken upstream or downstream of the atherosclerotic lesion from stable angina patients. The extent of stenosis and shear rates are calculated using computational angiographic imaging.

Shear, 3 helping to explain how platelet/H9251 main to enhance GPIb formation, and activation of (ranging from low to high physiologic shear) induce conformational activation of (see figure).2 This device, involving a rotating cone on a flat plate, applies uniform shear stress to a sample at shear rates ranging from low to high physiologic (~ 20-1600 s^-1) or pathologic levels (> 10 000 s^-1) in a stenotic artery. High shear induces conformational activation of the platelet receptor, glycoprotein Ib (GPIbα; the VWF-binding subunit of GPIb-IX-V), and VWF, leading to platelet activation, secretion of adenosine diphosphate (ADP), thromboxane A2 (TXA2) production, and activation of αIIbβ3. In this experimental setup, shear–induced platelet aggregation occurs without exposure to a thrombogenic surface, and GPIbα, VWF, ADP, and αIIbβ3 are indispensable.5 Recent studies reveal how shear stress can alter conformation of the GPIbα ligand-binding domain to enhance GPIbα–VWF bond strength,5 helping to explain how platelet adhesion and activation can occur at pathologic shear rates in flowing blood. Yong and colleagues also exposed blood from healthy individuals (collected from an arm vein) to shear rates of 1800 s^-1 for 5 minutes in a cone–plate viscometer and showed increased platelet P-selectin expression and platelet-monocyte interactions comparable with in vivo results (median wall shear rate, 1782 s^-1). However, while an inhibitory anti–P-selectin antibody completely blocked platelet-monocyte interaction, it did not affect the shear–induced increase in monocyte CD11b expression. CD11b (the α-subunit of the leukocyte integrin, αMβ2; CD11b/CD18; Mac-1) is up-regulated upon cellular activation and mediates adhesion to endothelial cells via intercellular adhesion molecule-1 (ICAM-1) or platelets via GPIbα.4,5 This finding suggests not only that platelet P-selectin mediates the interaction between platelets and monocytes, possibly involving its counterreceptor P-selectin glycoprotein ligand-1 (PSGL-1),6 but, in addition, that monocytes also respond to shear independent of platelet adhesion. It is known that shearing leukocytes alters PSGL-1 surface distribution to microvilli, facilitating cell rolling and attachment, as well as up-regulating CD18 expression.6

Recent studies suggest shear stress alone can provoke aggregation of discoid platelets unrelated to aggregation involving ADP/TXA2 secretion.7 The present study uniquely demonstrates platelet activation in vivo due to vascular stenosis, correlating with the extent of stenosis and levels of shear, but without a requirement for secondary platelet agonists (patients were treated with aspirin that blocks TXA2 production and clopidogrel that blocks the platelet ADP receptor, P2Y12). Despite no significant activation of αIIbβ3, this level of platelet activation could increase propensity for thrombosis and thrombotic risk, or else promote the inflammatory component of cardiovascular disease through activation of leukocytes (involving P-selectin or other platelet–derived proinflammatory factors).5 Increased P-selectin expression also implies release of other α-granule constituents, including PF4, adhesive proteins, mitogenic and angiogenic factors, coagulation factors, and fibrinolytic inhibitors.5 P-selectin is known to increase generation of tissue-factor–bearing, procoagulant microparticles from monocytes which can also increase thrombotic risk.8 Notwithstanding the potential technical and clinical challenges discussed by Yong et al, the findings raise many interesting mechanistic questions, and have significant clinical and therapeutic implications. Blood cells presumably circulate through the coronary vascular bed multiple times. How, then, can changes in activation be detectable in samples collected proximal and distal to the lesion that is, after a single passage? Are the shear–dependent effects on platelets and monocytes cumulative, reversible, or both, or is there a transient cellular “memory” of brief exposure to elevated shear? In this regard, it is known that platelets exposed to transient shear stress in the cone–plate viscometer retain the capacity to support VWF binding to GPIbα even after cessation of shear.9 Another possibility is that shear–exposed platelets are more susceptible to clearance from the circulation, through interactions with leukocytes or other cells, or through GPIbα-dependent interaction with αMβ2 on phagocytic cells, a mechanism involved in clearance of chilled platelets in the liver.10 Finally, a significant aspect of this study is what it reveals about conventional anti-platelet therapy (aspirin, clopidogrel). These drugs inhibit activation of αIIbβ3, and inhibition of αIIbβ3-dependent platelet aggregation under static or shear conditions represents the usual criteria for selection of
induced by high concentrations of FVIII (see figure, panel B). TLR7 and TLR9 are triggered by viral nucleic acids and the observed effects suggest that infections or vaccinations may potentially activate FVIII-specific memory B cells in patients with hemophilia A. Importantly, the observed effects are strictly dependent on the presence of FVIII. In the absence of FVIII, TLR9 triggering does not result in stimulation of FVIII–specific memory B cells (see figure, panel C). It is important to note that in the human model systems, TLR agonists as well as bystander T–cell help have been shown to result in polyclonal activation of human memory B cells independent of the presence of specific antigen. \(^6\) Based on these findings, it was hypothesized that TLR9 agonists or bystander T cells can drive the activation of human memory B cells resulting in their self-renewal and differentiation into antibody-secreting cells. \(^6\) However, findings in several murine models strongly suggest that—at least in mice—in vivo restimulation is strictly dependent on the presence of antigen. \(^6\) It remains difficult to extrapolate the results obtained by Allacher and coworkers to the human immune system. Low frequencies of circulating FVIII-specific memory B cells have been found in hemophilia A patients with inhibitors. \(^\) Presently, no data are available with respect to the impact of viral infections or vaccinations on the differentiation or proliferation of FVIII-specific human memory B cells. Therefore, it is currently not clear whether natural infections can enhance or reduce anamnestic antibody responses in patients with hemophilia A.

Unexpectedly, Allacher and coworkers show that high concentrations of the TLR9 agonist CpG–oligodeoxynucleotide (CpG-ODN) completely abolish the FVIII-driven restimulation of memory B cells (see figure, panel D). The authors speculate that modulation of dendritic cell function by high dosages of CpG-ODN induces regulatory CD4\(^+\) T cells that prevent the activation of CD4\(^+\) T cells required for restimulation of FVIII-specific memory B cells. The signaling pathways underlying the modulating effect of high dosages of CpG-ODN have not yet been clarified. Future studies in this area may help

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**REFERENCES**

1. Yong ASC, Pennings GJ, Chang M, et al. Intracoronary antiplatelet drugs (including α\(\_\)\(\_\)β\(_{\text{II}}\)\(\_\)β\(_{\text{III}}\)) inhibitors. Although blockade of TXA\(_2\), and ADP receptors P2Y\(_{\text{I}}\), and P2Y\(_{\text{I2}}\), inhibited shear-induced platelet P-selectin expression and platelet-monocyte aggregation in healthy donor samples in vitro, therapeutic doses of aspirin/clopidogrel did not block measurable shear-induced platelet activation (indicated by P-selectin expression) on passage through a stenotic artery in cardiovascular patients in vivo. \(^1\) Therefore, while antiplatelet drugs may reduce thrombotic risk, the present study emphasizes the need for a wider selection of drugs to attenuate pathologic platelet function. This novel analysis of shear–dependent effects on vascular cells in vivo also highlights the need for better bioassays of platelet function that take pathologic shear stress into account, and could lead to the identification of safer therapeutic targets.

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**THROMBOSIS & HEMOSTASIS**

**Comment on Allacher et al, page 259**

**Getting rid of bad memory**

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In this issue of Blood, Allacher and colleagues elegantly show that triggering of the innate immune system modulates the restimulation of preexisting FVIII-specific memory B cells in a murine model for inhibitor development in hemophilia A. \(^1\)

The development of inhibitory antibodies in patients suffering from the X-linked bleeding disorder hemophilia A provides a major side-effect of factor VIII (FVIII) replacement therapy. Pioneering studies by Hans Brackmann in Bonn showed that tolerance to FVIII can be restored by frequent administration of high dosages of FVIII, so-called immune tolerance induction (ITI) therapy. \(^2,3\) Despite its high overall success rate, the mechanisms underlying successful ITI have remained obscure for a long time. An earlier landmark study from the Reipert group revealed that high dosages of FVIII prevent the restimulation of FVIII-specific memory B cells in hemophilic mice. \(^4\) This observation suggested that administration of high dosages of FVIII eliminates FVIII-specific memory B cells thereby contributing to the decline in FVIII inhibitor levels during ITI. In the present report, the same group explores whether triggering of pattern recognition receptors of the innate immune system modulate the restimulation of FVIII-specific memory B cells. Triggering of Toll-like receptors TLR7 and TLR9 effectively promoted the restimulation of FVIII-specific memory B cells at a low concentration of FVIII (see figure, panel A). Triggering of TLR7 and TLR9 also abolished the inhibition of memory responses

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