blood commentary

THROMBOSIS AND HEMOSTASIS

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Btk inhibitors in atherosclerosis

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In this issue of *Blood*, Busygina et al provide provocative evidence that there is a potential role for Bruton tyrosine kinase (Btk) inhibitors in inhibiting platelet aggregation specifically at the site of unstable atherosclerotic plaques.[1]

Antiplatelet therapies are a mainstay for the prevention of atherosclerotic complications, particularly for secondary prevention of subsequent events in both the culprit and nonculprit vessels.[2] Over the past several decades, there has been a transition in clinical practice to preferentially using antiplatelet over antithrombotic therapies for the chronic prevention of atherosclerotic events because of the recognition that platelet activation and aggregation are the key events in acute arterial thrombosis that occurs when plaques undergo rupture or erosion and the superior safety margin with regard to major bleeding. There is also increasing evidence that even in the absence of plaque rupture, platelet-endothelial interactions may be important in accelerated plaque progression.[3]

Efficacy of antiplatelet therapy can be modified by blocking different aspects of platelet function (eg, inhibitors of cyclo-oxygenase, adenosine 5'-diphosphate [ADP] receptors, protease-activated receptors, and $\alpha_{IIb}\beta_3$ integrin). The highly varied approaches for selecting antiplatelet therapy are based on the need to balance risk of bleeding with risk of arterial thrombosis in different patient categories (eg, coronary vs noncoronary disease, stable vs unstable phase, diabetic vs nondiabetic). In any patient category, preventing platelet adhesion and aggregation at the site of plaque rupture while preserving normal hemostatic function in hemorrhagic conditions such as trauma or aneurysm leak would be advantageous. The study by Busygina et al proposes that this could be possible with oral inhibitors of Btk. This concept is based on the known effects of Btk on platelet activation signaling from ligation of cell-surface adhesion molecules including glycoprotein VI (GPVI) and GPIb.[4] Aggregometry has revealed that ibrutinib inhibits collagen (ie, GPVI-mediated) but not ADP-mediated platelet aggregation,[5] which in part explains the increased bleeding with ibrutinib independent of thrombocytopenia, particularly in patients already receiving standard antiplatelet or antithrombotic therapy.[6] Using high-throughput in vitro models, Busygina et al demonstrate that oral Btk inhibitors prevent platelet accumulation on exposed human plaque components selectively through GPVI signaling. Although it is already known that GPVI plays a critical role in platelet recruitment at sites of vascular injury,[7] the implication of the current study is that aggregation can be reduced by inhibition of outside-in signaling by GPVI.

The observation that Btk inhibitors had a preferential effect in inhibiting platelet aggregation on plaque components rather than normal collagen was attributed to preservation of non-GPVI platelet collagen receptors (integrin $\alpha_2\beta_1$) that are less selective for altered collagen within plaque. However, the notion that ibrutinib is safe must be tempered by known bleeding complications in oncology patients. One could argue that bleeding is a so-called price of doing business in secondary prevention and that selective inhibition of platelet-plaque interactions may be particularly useful in particularly high-risk settings such as iatrogenic plaque disruption in patients undergoing percutaneous mechanical revascularization.

There are some unsolved issues that must be addressed before directly applying results of this study in patients. The data provided on shear dependency of Btk inhibitors are insightful and informative with regard to effect in different vessel types. However, flow in large arteries is pulsatile, with low diastolic shears in noncoronary vessels, and shear in regions of plaque can be spatially heterogeneous and even oscillatory.[8] Although platelet accumulation on plaque homogenates or sections is a reasonable ex vivo model, binding to collagen may not be entirely reflective of events that occur from disruption of nondiseased vessels. Another important consideration in humans is whether plaque-specific antiplatelet actions of Btk inhibitors could increase microhemorrhage within plaque neovessels, which has been implicated in plaque progression and risk for events.[9] An additional consideration is the increased risk of cardiac arrhythmias, particularly a fourfold increase in risk of atrial fibrillation, with ibrutinib.[10]

The study by Busygina et al adds to our knowledge of the complex interplay between tyrosine kinase inhibitors and risk of cardiovascular disease. Whereas other kinase inhibitors (eg, ponatinib and nilotinib) have been associated with vascular events, the current study by Busygina et al implies a potentially beneficial role for Btk inhibitors as antiplatelet agents. The study also provides reassurance that the field of cardio-oncology can yield information beyond toxic effects of chemotherapeutics that may benefit patients with atherosclerotic disease, similar to the evolution of macrolide-coated coronary stents, which are now routinely used to inhibit adverse neointimal proliferation.

REFERENCES

1. Busygina K, Jamasbi J, Seiler T, et al. Oral Bruton tyrosine kinase inhibitors selectively block atherosclerotic plaque–triggered thrombus formation in humans. *Blood*. 2018; 131(24):2605-2616.

2. Steg PG, Dorman SH, Amarenco P. Atherothrombosis and the role of antiplatelet therapy. *J Thromb Haemost*. 2011;9(9 suppl 1): 325-332.

3. Shim CY, Liu YN, Atkinson T, et al. Molecular imaging of platelet-endothelial interactions and endothelial von Willebrand factor in early and mid-stage atherosclerosis. *Circ Cardiovasc Imaging*. 2015;8(7):e002765.

4. Quek LS, Bolen J, Watson SP. A role for Bruton's tyrosine kinase (Btk) in platelet activation by collagen. *Curr Biol*. 1998;8(20): 1137-1140.

5. Kamel S, Horton L, Ysebaert L, et al. Ibrutinib inhibits collagen-mediated but not ADP-mediated platelet aggregation. *Leukemia*. 2015;29(4):783-787.

6. Shatzel JJ, Olson SR, Tao DL, McCarty OJT, Danilov AV, DeLoughery TG. Ibrutinib-associated bleeding: pathogenesis, management and risk reduction strategies. *J Thromb Haemost*. 2017;15(5):835-847.

7. Massberg S, Gawaz M, Grüner S, et al. A crucial role of glycoprotein VI for platelet recruitment to the injured arterial wall in vivo. *J Exp Med*. 2003;197(1):41-49.

8. Nigro P, Abe J, Berk BC. Flow shear stress and atherosclerosis: a matter of site specificity. *Antioxid Redox Signal*. 2011;15(5):1405-1414.

9. Virmani R, Kolodgie FD, Burke AP, et al. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. *Arterioscler Thromb Vasc Biol*. 2005;25(10): 2054-2061.

10. Brown JR, Moslehi J, O'Brien S, et al. Characterization of atrial fibrillation adverse events reported in ibrutinib randomized controlled registration trials. *Haematologica*. 2017;102(10):1796-1805.

LYMPHOID NEOPLASIA

Comment on Ren et al, page 2670

HBV messing with the B-cell genome leads to DLBCL

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In this issue of *Blood*, Ren et al report the results of a broad genomic and transcriptomic analysis of hepatitis B virus (HBV)–associated diffuse large B-cell lymphomas (DLBCLs) in Chinese patients, providing for the first time a distinctive molecular profile of these tumors.[1]

Among the 275 DLBCL samples characterized by whole-genome sequencing and whole-exome sequencing, 20% were from HBV infection surface antigen–positive (HBsAg+) patients. This high proportion, related to endemic HBV in China, provides a unique opportunity for investigating differences in clinical and mutational spectra between HBV-related and -unrelated DLBCL in the context of a common ethnic background. HBsAg+ DLBCL patients compared with HBsAg− patients had significantly younger age, more aggressive disease, and shorter survival, similar to the findings in another study in Chinese patients.[2] Genome-wide analysis revealed an increased total mutation load in HBsAg+ DLBCL possibly resulting from APOBEC enzyme activity and also a distinctive set of mutated genes that might be related to the activity of the B-cell–specific activation-induced cytidine deaminase (AID). *BCL6* was the lymphoma-related gene most frequently mutated (79%) in HBsAg+ DLBCL, together with other genes involved in the FOXO signaling pathway (*CXCR4*, *KLF2*, and *SGK1*). The authors speculate that alterations of the FOXO pathway might promote the development of HBsAg+ DLBCL by inducing antigen-independent tonic B-cell receptor signaling, and they suggest BCL6 as an important therapeutic target.

HBV and hepatitis C virus (HCV) share hepatotropism and the capacity to induce B-cell lymphomas.[3] In the case of HCV, it is generally agreed that lymphomas develop as a consequence of the protracted stimulation of B cells expressing specific stereotyped idiotypes putatively directed to a viral antigen that has not yet been identified.[4] A major argument in favor of this hypothesis is the frequent regression of indolent lymphomas associated with HCV, but not with HCV+ DLBCL, after the clearance of infection with antiviral therapy.[5] Ren et al characterized the V(D)J region of immunoglobulin (Ig) heavy chain in 15 HBsAg+ DLBCL samples and were unable to identify recurrent stereotyped idiotypes. This contrasts with another study[2] in HBsAg+ DLBCL Chinese patients that identified 2 stereotyped sequences in 4 of 16 patients studied and claimed significant homology of these antibodies to antibodies specific for HBsAg.

On the basis of their results, Ren et al rejected the hypothesis that HBV, like HCV, causes lymphomas by protracted antigenic stimulation of B cells that produce virus-specific antibodies and suggest that infection of B cells by HBV may induce a hyperactive status that leads to enhanced mutagenesis mediated in part by APOBEC and AID. Indeed, these 2 hypotheses might not necessarily be mutually exclusive. In the case of HCV, for which an antigen-driven mechanism of lymphomagenesis is generally accepted, it has also been shown[6] that infection of B cells by the virus induces a mutator phenotype that leads to a five- to 10-fold increase in mutation frequency in *BCL6*, *Ig heavy chain*, and *p53* genes. Conversely, it is also known that some HBV-infected individuals develop type 2 mixed cryoglobulinemia, a monoclonal B-cell lymphoproliferative disorder that can regress after infection is suppressed by antiviral therapy.[7] This suggests antigenic pressure as the cause of monoclonal lymphoproliferation because that is the case for HCV-related mixed cryoglobulinemia. HCV causes a large spectrum of lymphoproliferative disorders ranging from the most frequent mixed cryoglobulinemia, which is benign but highly prone to neoplastic evolution, to indolent lymphomas mainly originating from marginal zone B cells, to aggressive DLBCL. This suggests that evolution from benign monoclonal lymphoproliferation to aggressive lymphoma may be driven by continual antigenic stimulation and accumulation of mutations. By contrast, HBV rarely causes mixed cryoglobulinemia, and HBV-related indolent lymphomas are much rarer than