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PLATELETS AND THROMBOPOIESIS

Comment on Davizon-Castillo et al, page 727

Platelet hyperreactivity: a new twist in old mice

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In this issue of *Blood*, Davizon-Castillo et al established a causative link between platelet hyperreactivity in old mice and increased systemic levels of tumor necrosis factor α (TNF-α), which functions via megakaryocyte metabolic reprogramming that leads to platelet mitochondrial dysfunction.[1]

Platelet hyperreactivity is not merely an inevitable adverse effect of conditions ranging from infections to diabetes, it is a strong contributing factor that exacerbates most aging-related human pathologies. Large-scale and long-term projects, such as the Framingham Heart Study, demonstrated that even in patients free of cardiovascular disease, intrinsic platelet reactivity is associated with future arterial and venous thrombosis.[2] Taken together, recent studies indicate that platelet reactivity is not just a biomarker of adverse effects, it is by itself a condition that requires management. This is not limited to cardiovascular disease and stroke because antiplatelet strategies have also been successfully used to slow down cancer progression and associated morbidities.[3] Thus, understanding the underlying causes and the modifying factors of platelet reactivity are becoming more important.

The previously described mechanisms leading to platelet hyperreactivity include genetic variations in platelet proteins such as surface receptors or changes in the balance between procoagulant and anticoagulant factors in plasma. Regardless of the relative importance of each factor separately, platelet reactivity is likely multifactorial in aging humans and is a result of numerous factors combined that multiply exponentially with age. For instance, most age-related pathologies, from cancer to inflammation, are often accompanied by dyslipidemia and increased oxidative stress. The latter condition leads to the accumulation of oxidized phospholipid products in blood that directly activate platelets by engaging their Toll-like and scavenger receptors.[4,5] Likewise, chronic inflammation and platelet reactivity represent another vicious circle that increases the risk of thrombosis and of snowballing into serious adverse effects. New therapeutic strategies that allow age-related platelet hyperreactivity to be managed might substantially slow down progression of a number of chronic diseases associated with severe morbidities in aging patients.

Despite the well-documented and acknowledged link between inflammation and platelet reactivity, the exact molecular mechanisms underlying platelet reactivity in inflammation are not well understood. Proinflammatory cytokines, particular TNF-α, are considered to be an important link between inflammation and the prothrombotic state in pathological conditions associated with inflammation. Increased levels of TNF-α correlate with the prothrombotic state in several pathologies. In vivo studies have directly implicated TNF-α in enhanced thrombosis in inflammation.[6] In addition, in vitro studies have shown that TNF-α promotes platelet activation and aggregation induced by physiological platelet agonists such as collagen and thrombin.[7]

In their study, Davizon-Castillo et al investigated the mechanism of increased platelet reactivity and thrombosis in old mice. The authors demonstrated that in old mice, plasma levels of TNF-α, platelet numbers, and platelet responses to physiological agonists were increased in both ex vivo and in vitro settings. Remarkably, increased platelet responses were observed in washed platelets (ie, in the absence of prothrombotic plasma components, including TNF-α). Similar results were obtained in a comparative analysis of human platelets from younger and older adults. These results are even more striking considering that 60% of the older adults were taking aspirin. Thus, it seemed that aging affects not only platelet counts but also their intrinsic characteristics, possibly during megakaryopoiesis and thrombopoiesis.

Detailed analysis of the murine megakaryocyte transcriptome revealed changes in several signaling pathways, including mitochondrial function, oxidative phosphorylation and TNF-dependent pathways, pointing toward a rather unique and promising role of metabolism in platelet reactivity. Indeed, platelets from older mice contained increased numbers of mitochondria, displayed increased oxygen consumption by mitochondria, and generated more adenosine monophosphate, adenosine diphosphate, and adenosine triphosphate. The authors hypothesized that these changes were induced by increased TNF-α and are responsible for age-associated platelet hyperreactivity.

The key role for TNF-α was underscored by the fact that chronic TNF-α treatment of younger mice resulted in characteristics that practically mirrored those of old mice on the basis of comparisons of their megakaryocyte transcriptomes. It seems that TNF-α alone might account for several platelet characteristics in aging, as shown in a series of elegant rescue experiments that ultimately strengthened the causative connections between TNF-α and platelet reactivity. Whereas chronic treatment of young mice with TNF-α exacerbated platelet reactivity, TNF-α blockade in old mice reversed platelets' pathological

traits, including increased mitochondrial mass, thereby alleviating platelet hyperreactivity. These and other approaches convincingly showed that TNF-α induces metabolic reprogramming in megakaryocytes and platelets, which likely contributes to the platelet-dependent complications of aging.

The authors also demonstrate that it is very likely that the TNF-α–dependent mechanism of platelet reactivity might operate in other pathological situations, such as in myeloproliferative neoplasms, characterized by an increase in both TNF-α and thrombosis. Thus, TNF-α seems to be an attractive target for the treatment of inflammation, neoplasms, and age-related platelet hyperreactivity. Several clinical studies on TNF inhibitors[8] reported a reduced risk of myocardial infarction and even insulin resistance, the benefits of which might be explained by the results of the Davizon-Castillo et al study. It will be important to assess the safety of TNF-α inhibitors for the long-term treatment of aging patients. At present, known adverse effects of TNF-α inhibitors, which were used in clinical practice for many years to treat rheumatoid diseases, include infections, autoimmune problems, and even possible effects on malignancy, and they have been described in the most recent reviews.[9,10] An alternative strategy is to focus more precisely on megakaryopoiesis and platelet metabolism, which may be a safer strategy for long-term treatment. In any scenario, the study by Davizon-Castillo et al provides interesting new perspectives and opportunities for improving the management of platelet hyperreactivity and thrombosis associated with aging.

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TRANSPLANTATION

Comment on Sinha et al, page 776

APC ameliorates pulmonary complications in cGVHD

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In this issue of *Blood*, Sinha et al demonstrated that activated protein C (APC) reduced the severity of pulmonary complications in a murine model of chronic graft-versus-host disease (cGVHD). The protective effect of APC against cGVHD was mediated by a biased signaling of protease-activated receptor 1 (PAR1) on T lymphocytes.[1]

cGVHD causes morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT) and occurs with various degrees of severity in 30% to 70% of recipients.[2] In addition to the organs usually targeted by acute GVHD (aGVHD) (skin, liver, and gastrointestinal tract), cGVHD can affect lungs, mouth, esophagus, joints, muscles, fasciae, eyes, hair, nails, and genitalia. Fibrotic changes in the lungs can result in bronchiolitis obliterans (BO), which is characterized by thickening of the bronchial wall, narrowing of the bronchial lumen, and trapping of air. The pathophysiology of cGVHD involves an early inflammatory phase induced by tissue injury from aGVHD, cytotoxic drugs, and infections. The early inflammation is mediated by innate immunity, which initiates an adaptive immune response that includes B lymphocyte hyper-responsiveness and Th17 differentiation of $CD4^+$ T lymphocytes. This early phase is followed by chronic inflammation and dysregulation of adaptive immunity. Thymic lesions disrupt negative selection of T cells (central tolerance). Reduction in regulatory T (Treg) cells, T follicular regulatory (TFR) cells, regulatory B cells, and regulatory natural killer cells diminish peripheral tolerance. Expansion of B cells in germinal centers (GCs) assisted by T follicular helper (TFH) cells results in the production of pathogenic auto- or alloantibodies. The final phase of cGVHD is associated with fibrosis in various organs.[2]

GVHD, particularly steroid-refractory GVHD, is associated with endothelial loss of thrombomodulin (TM).[3] Preclinical studies showed that soluble TM reduces the severity of GVHD.[4] Thrombin binds to TM and activates protein C bound to the endothelial protein C receptor. APC acts as a natural anticoagulant by cleaving activated factor V and factor VIII. However, APC can also cleave protease-activated receptors (PARs)[5] (see figure). Cleavage of PARs by APC does not affect the coagulation cascade, but rather results in cytoprotective effects.[5] Recombinant mutant APC molecules that are unable to bind factor Va (by replacing lysine residues