Aging is intrinsically linked with physical decline and is a major risk factor for a broad range of diseases including cardiovascular disease and thrombosis. There is growing appreciation for the role of platelets as pathological mediators of age-related thrombosis. It is well documented that platelet hyperactivity is a key alteration associated with aging that appears to be driven in part by oxidative and metabolic pathways (1,2). In addition, chronic inflammation caused by increased local and systemic mediators has been shown to accompany the aging process and can further contribute to vascular disease and thrombosis. Nevertheless, a clear mechanistic link between inflammation and platelet hyperreactivity during aging is not well established. In the August 29th, 2019 issue of Blood (3), a study by Davizon-Castillo et al. provide compelling evidence that age-associated inflammation promotes platelet activity and platelet thrombi formation. Using a well-designed cross-sectional study in mice and humans the authors reported a critical role of TNF-α as a proinflammatory mediator in platelet hyperreactivity of aging. Blood 2019;134:727-40.

To assess the relationship between TNF-α and platelet reactivity, the authors utilized several different murine models of TNF-α elevation or depletion. They demonstrated that daily injections of young mice with TNF-α, which increased plasma TNF-α to a level similar to aged mice, closely recapitulated the platelet hyperreactivity of aged mice. Similar results of platelet hyperreactivity was found using a genetic murine model of chronically elevated TNF-α (TNFΔARE). The authors then performed in vivo neutralization studies using a monoclonal anti-TNF-α antibody that significantly lowered plasma TNF-α levels and decreased platelet activation responses in aged mice similar to the levels observed in young mice. Furthermore, injection of TNF-α into young p55/p75 KO mice (deficient for both TNF-α receptors) did not increase platelet activation responses suggesting a direct role of TNF-α in platelet activation. One limitation of these studies is that the platelet activation and adhesion assays were performed ex vivo. In vivo thrombosis models would provide more physiological relevance to establish TNF-α as a mediator of thrombosis. Additionally, it remains unclear whether these
mechanistic findings can be translated to human aging, so future studies should consider designing experiments to test these possibilities in humans.

To assess what is driving the hyperactivity of platelets during aging, the authors evaluated the bone marrow compartment and specifically focused on megakaryocytes. Immunophenotypic analysis identified skewed megakaryocyte progenitor populations in aged mice. Subsequent examination of the megakaryocyte transcriptome by single cell RNA-sequencing revealed transcriptional alterations in distinct subpopulations of megakaryocytes that corresponded with changes in mitochondrial function, oxidative phosphorylation, and inflammatory signaling pathways, indicating an intriguing role of mitochondria in platelet hyperreactivity during aging. Indeed, platelets from aged mice showed altered bioenergetics reflected by increased oxygen consumption, higher ATP at baseline and metabolomic profiling showing decrease in glycolysis. In addition, electron microscopy showed that platelet mitochondrial mass was increased in aged mice.

Given that mitochondrial and TNF signaling pathways were both overrepresented in megakaryocytes from old mice, the authors examined the role of TNF-α on the platelet mitochondrial profile. Chronic systemic exposure of young mice to TNF-α was shown to increase the platelet mitochondrial mass. Moreover, the megakaryocyte transcriptome was altered similarly to that of aged mice suggesting that the effects of TNF-α are likely driven by its action on megakaryopoiesis and thrombopoiesis. Using a neutralizing antibody to abrogate TNF-α dependent signaling, the authors rescued the increase in mitochondrial mass of aged mice. Overall, these experiments suggest that TNF-α is influencing platelet mitochondria, but the mechanistic relationship remains unclear. To strengthen the evidence for TNF-α as a pathologic mediator, additional studies could examine the mechanisms of altered TNF-α on platelet metabolomics and mitochondrial bioenergetics. Furthermore, the authors did not establish a clear mechanistic link between mitochondrial dysfunction and platelet hyperactivity. Future studies should incorporate approaches to examine how alterations in platelet metabolomics and mitochondrial bioenergetics induces aberrant platelet hyperactivity during aging.

In light of the accumulating evidence for diverse functional roles of mitochondria in platelets (4), these findings provide novel directions for future studies. For example, the increases in platelet mitochondrial mass observed in aged mice could be caused in part by decreased mitochondrial turnover and decline in mitophagy with age. Phagosome maturation was one of the top pathways identified by Ingenuity Pathway Analysis that was altered between old and young mice, and recent studies have shown that autophagy/mitophagy is important for maintaining platelet functional capacity by protecting it from oxidative stress-mediated mitochondrial damage (5,6). Several lines of evidence indicate that aging impairs mitophagy and prevents removal of dysfunctional or damaged mitochondria (7) although its specific role in platelet activation in the context of aging is not well understood. On the other hand, it has been demonstrated that activated platelets can release mitochondria and mitochondrial DNA which can promote inflammatory mediators and may further induce platelet activation (8-10). It would be interesting to see whether more mitochondria and mitochondrial DNA are released by hyperreactive platelets during aging that may potentiate inflammatory and thrombotic responses.

TNF-α is known to be elevated in a multitude of pathologic conditions in addition to aging. Many of the experimental results by Davizón-Castillo and colleagues indicate that the effects of TNF-α on megakaryocytes and platelets may be broadly applicable to other pathological conditions with elevated TNF-α. Indeed, the authors showed that platelets are hyperreactive from patients with JAK2 V617F-positive myeloproliferative neoplasms, a clonal hematologic disease associated with elevated TNF-α and characterized by thrombocytosis and increased thromboembolic risk (11). In a broader context, JAK2 is a common candidate driver mutation in clonal hematopoiesis of indeterminant potential (CHIP), a recently discovered age-related hematologic condition that increases the risk for hematologic malignancies and cardiovascular disease (12,13). Previous studies have shown that somatic mutations that drive clonal expansion of hematologic cells cause reprogramming of macrophages to a more inflammatory phenotype that play a causal role in cardiovascular disease (14,15). It would be interesting to examine the effects of age-related clonal hematopoiesis on megakaryocytes and platelets and their contributions to thrombotic vascular disease in future studies.

Overall, Davizón-Castillo and colleagues provide considerable evidence that TNF-α is a key driver of megakaryocyte alterations and platelet hyperreactivity that may contribute to thrombotic risk during aging. Given that TNF-α inhibitors are approved and widely used to slow progression of autoimmune conditions such
as rheumatoid arthritis, it seems to be an attractive target for the development of therapeutic strategies against age-related comorbidities including thrombosis. Aging is associated with a multitude of comorbidities including cancer, cardiovascular, autoimmune, and infectious disease. A major challenge is to dissect the interactions between different inflammatory and prothrombotic factors and their relative contributions to age-related diseases and ultimately to identify pharmaceutical targets with minimal side effects. The Davison-Castillo paper provides a framework to target TNF-α or factors downstream of its signaling pathways as a potential therapeutic strategy for the prevention and management of age-associated thrombotic complications.

Acknowledgments

Funding: This work was supported by funding from the National Institute of Health AG049784 to S.D., and HL007344 to S.X.G., and from the American Heart Association 18IPA34180014 to S.D.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References


doi: 10.21037/aob.2020.03.01

Cite this article as: Gu SX, Dayal S. Inflammation mediated platelet hyperactivity in aging. Ann Blood 2020.