Editorial Commentary

Inflammation mediated platelet hyperactivity in aging

Sean X. Gu, Sanjana Dayal

Department of Internal Medicine, The University of Iowa Carver College of Medicine, Iowa City, IA, USA

Correspondence to: Sanjana Dayal, PhD, FAHA. Department of Internal Medicine, The University of Iowa Carver College of Medicine 3186 Med Labs, 500 Newton Street Iowa City, IA 52242, USA. Email: sanjana-dayal@uiowa.edu.

Comment on: Davizon-Castillo P, McMahon B, Aguila S, *et al.* TNF-α-driven inflammation and mitochondrial dysfunction define the platelet hyperreactivity of aging. Blood 2019;134:727-40.

Received: 07 February 2020; Accepted: 02 March 2020; Published: 25 March 2020.

doi: 10.21037/aob.2020.03.01

View this article at: http://dx.doi.org/10.21037/aob.2020.03.01

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 activation during aging.

The authors utilized several complementary approaches. Studies in murine models showed that aged mice have elevated plasma TNF-α levels and they exhibit increased platelet reactivity and accelerated platelet thrombi formation *ex vivo*. Interestingly, similar results were observed with human platelets isolated from volunteers despite over 60% of older individuals (versus less than 10% of younger individuals) were receiving aspirin at the time of sample collection. It is noted that about 72% of older individuals were on statins but the co-morbidities of the study population were not listed. Many age- related co-morbidities such as atherosclerosis, obesity, hyperlipidemia, etc. are known to be associated with an increased inflammatory state and thrombotic complications. Given that aging is a complex and multifactorial process, inclusion of samples only from healthy individuals will be a better study design to assess the effects of aging alone on platelets that would eliminate many of the confounding factors associated with the aged population.

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.

© Annals of Blood. All rights reserved.

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 Davizon-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, Davizon-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

© Annals of Blood. All rights reserved.

relative contributions to age-related diseases and ultimately to identify pharmaceutical targets with minimal side effects. The Davizon-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

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Yuwei Zhao, PhD (Research fellow of Blood Research Laboratory, Chengdu Blood Center, Chengdu, China).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at: http://dx.doi.org/10.21037/aob.2020.03.01). 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.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: https://creativecommons.org/licenses/by-nc-nd/4.0/.

References

1. Dayal S, Wilson KM, Motto DG, et al. Hydrogen peroxide promotes aging-related platelet hyperactivation and thrombosis. Circulation 2013;127:1308-16.
2. Yang J, Zhou X, Fan X, et al. mTORC1 promotes aging-related venous thrombosis in mice via elevation of platelet volume and activation. Blood 2016;128:615-24.
3. Davizon-Castillo P, McMahon B, Aguila S, et al. TNF-α-driven inflammation and mitochondrial dysfunction define the platelet hyperreactivity of aging. Blood 2019;134:727-40.
4. Melchinger H, Jain K, Tyagi T, et al. Role of Platelet Mitochondria: Life in a Nucleus-Free Zone. Front Cardiovasc Med 2019;6:153.
5. Ouseph MM, Huang Y, Banerjee M, et al. Autophagy is induced upon platelet activation and is essential for hemostasis and thrombosis. Blood 2015;126:1224-33.
6. Lee SH, Du J, Stitham J, et al. Inducing mitophagy in diabetic platelets protects against severe oxidative stress. EMBO Mol Med 2016;8:779-95.
7. Jang JY, Blum A, Liu J, et al. The role of mitochondria in aging. J Clin Invest 2018;128:3662-70.
8. Boudreau LH, Duchez AC, Cloutier N, et al. Platelets release mitochondria serving as substrate for bactericidal group IIA-secreted phospholipase A2 to promote inflammation. Blood 2014;124:2173-83.
9. Zhao Z, Wang M, Tian Y, et al. Cardiolipin- mediated procoagulant activity of mitochondria contributes to traumatic brain injury- associated coagulopathy in mice. Blood 2016;127:2763-72.
10. Zhong Z, Liang S, Sanchez-Lopez E, et al. New mitochondrial DNA synthesis enables NLRP3 inflammasome activation. Nature 2018;560:198-203.
11. Fleischman AG, Aichberger KJ, Luty SB, et al. TNFalpha facilitates clonal expansion of JAK2V617F positive cells in myeloproliferative neoplasms. Blood 2011;118:6392-8.
12. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med 2014;371:2488-98.
13. Genovese G, Kahler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med 2014;371:2477-87.
14. Fuster JJ, MacLauchlan S, Zuriaga MA, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. Science 2017;355:842-7.
15. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. N Engl J Med 2017;377:111-21.

doi: 10.21037/aob.2020.03.01
Cite this article as: Gu SX, Dayal S. Inflammation mediated platelet hyperactivity in aging. Ann Blood 2020;5:10.

© Annals of Blood. All rights reserved.