Editorial Commentary

Aging, chronic inflammation, and platelet hyperactivity

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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).

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: 06 April 2020; Accepted: 20 April 2020; Published: 30 June 2020.

doi: 10.21037/aob-20-32

View this article at: http://dx.doi.org/10.21037/aob-20-32

Platelets are small anucleate cells in blood, which play key roles in hemostasis and thrombosis (1). After red blood cells, platelets are the most abundant cells in circulation. Although the prevailing view is that platelets are produced from the megakaryocytes in the bone marrow, a recent study has shown that they can also be produced from megakaryocytes in the lungs (2). Megakaryopoiesis and platelet generation involve a series of well-orchestrated cellular processes including commitment of hematopoietic stem cells, differentiation and proliferation of megakaryocytic progenitors, and maturation and shedding of megakaryocytes to produce functional platelets (3). Several cytokines and growth factors in the bone marrow such as thrombopoietin, interleukin-1 (IL-1), IL-6, and IL-11 have been reported to contribute to these processes, which can be further regulated by the fluctuations of cytokines and growth factors during aging or pathological states (1,3). Interestingly, although platelets lack a nucleus, they are prepackaged with the residual RNA, organelles and transcriptional machinery that make them capable of *de novo* synthesis in response to environmental stimuli (4,5). In addition to their roles in hemostasis and thrombosis, mounting evidence reveals that platelets are versatile cells and are significantly involved in numerous physiological and pathological pathways, such as inflammation and immune responses, angiogenesis, atherosclerosis, lymphatic vessel development, liver regeneration, and tumor metastasis (1). Platelet disorders are therefore associated with many human diseases, such as cardiovascular diseases, cancer, renal diseases, and microorganism infections (6-8).

In the article published in *Blood* (9), Davizon-Castillo *et al*. elegantly demonstrated that age-related pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α) drives megakaryocyte metabolic reprogramming, platelet mitochondrial dysfunction and platelet hyperreactivity. There is a long-standing link between aging, chronic inflammation and platelet activity. However, no causal evidence exists to directly link age-related TNF-α upregulation to platelet hyperreactivity. Davizon-Castillo *et al.* showed that washed platelets from old mice exhibited increased activation of platelet integrin αIIbβ3, phosphatidylserine exposure, platelet aggregation and enhanced thrombus formation under flow conditions. Remarkably, exogenous administration of TNF-α to young mice recapitulated the aged megakaryocyte and platelet phenotype. TNF-α blockade with a monoclonal anti-TNF-α antibody in older mice reversed platelet hyperreactivity, thrombosis, and mitochondrial changes. Additionally, they used $TNF^{\Delta ARE}$ mice, which have chronically elevated levels of TNF-α, and mice lacking functional TNF receptors (p55/

p75 KO mice) to further demonstrate their observations. These results establish the causative link between increased systemic levels of TNF-α and platelet hyperreactivity in old mice. The discovery is further supported by the observation of increased mitochondrial mass in human patients with myeloproliferative neoplasms, which is associated with higher TNF-α levels and platelet hyperreactivity. The authors observed an alteration of inflammatory signaling pathways, including TNF-α, IL-1ß and IL-6, in aged mice determined by single cell RNA-sequencing of primary bone megakaryocytes. Interestingly, exogenous administration of IL-1ß did not promote platelet hyperreactivity. As previous evidence has shown that IL-6 can regulate megakaryocyte formation, platelet generation, platelet function, and IL-6 and TNF-α overlap in downstream transcriptional response, it is worthwhile to further characterize the role of IL-6 in this process (10). In addition, as both TNF-α and IFN-γ are cytokines in Th-1 like immune response, it would be also interesting to examine whether IFN-γ blockage has the similar effect as the anti-TNF-α antibody.

Platelet activation is an energy-dependent process, but the type and integrated use of metabolic fuels required to participate are still poorly understood. Previously, it has been reported that activated platelets shift to a predominantly glycolytic phenotype coupled with a minor rise in mitochondria oxygen consumption (11). Other groups have further found that platelets switch to aerobic glycolysis and accelerate flux through the pentose-phosphate pathway (PPP) during activation (12). In this paper, Davizon-Castillo *et al.* used liquid chromatography mass spectrometry-based metabolomics to study the systemic metabolic profiles in platelets from young and old mice. Interestingly, they found that platelets from old mice had depressed late-stage glycolytic metabolites pyruvate and lactate. Meanwhile they also revealed that platelets from old mice have a higher ATP-linked respiration capacity after activation, and elevated PPP intermediaries 6PGL, 6PG and E4P. However, this distinctive metabolic profile has only been observed in aged mice; the same experiment in TNF-α treated mice has not been performed, making it unclear whether the metabolic reprogramming is caused by TNF-α, other age-related factors, or both. Moreover, the authors interpreted the elevation of the PPP as a compensatory response to oxidative stress or accelerated cellular proliferation. In most cells, the PPP is the major source of NADPH production, which is essential for scavenging reactive oxygen species (ROS). In immune cells, NADPH from the PPP is used as a substrate for the enzyme NADPH oxidase (NOX) to generate ROS. Platelets, which are now emerging in recognition as immune cells, have also been reported to express NOX (13). NOX-generated ROS has been shown to regulate αIIbβ3-integrin activation (14) and contributes to platelet activation in aging (15). Based on the observed elevation of PPP and the contradicting role of PPP-NADPH-ROS axis in platelets compared to other cells, it is worthwhile to check levels of NADPH and ROS in aged and TNF-α treated mice, and explore the possibility of ROS serving as a missing link between metabolism reprogramming and hyperreactivity. In addition to serving as an energy and metabolite provider, mitochondria contribute to platelet activation by other functions such as mitochondrial permeability transition, collapse of mitochondrial membrane potential (1Ψ m), Ca^{2+} homeostasis and apoptosis (16). Whether these functions are affected during aging remains to be further elucidated.

Platelet hyperreactivity has been associated with many diseases such as cardiovascular diseases, cancer, sickle cell disease, inflammatory bowel disease, sepsis, rheumatoid arthritis, myeloproliferative disease, Alzheimer's disease and diabetes (1,7,17-19), however, the exact mechanisms are still not very clear. Whether the same pathway discovered by Davizon-Castillo *et al.* contributes to these disease processes requires further study. Deemed inflammatory cells decades ago, platelets store and release a substantial repertoire of inflammatory mediators upon activation that attract key immune cells, enhancing inflammation (20). Thus, platelets are a pivotal player in the pathogenesis of multiple inflammatory diseases. As chronic inflammation induces platelet hyperreactivity, platelets may in turn accelerate inflammation, making platelets a promising target to manipulate the inflammatory response in diseases. Notably, COVID-19, an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) that has spread to all 7 continents with over 200 countries and territories, is also associated with a high inflammatory burden and cytokine storm. Researchers found that TNF-α, IL-6, IL-10 and IL-2R are closely related to severity of COVID-19 (21). Disseminated intravascular coagulation (DIC) as well as micro-thrombosis were also common in COVID-19 related deaths (22). As reported, old age is one of the predisposing conditions for COVID-19 pneumonia (21). It is possible that platelet hyperreactivity contributes to DIC and higher COVID-19 incidence/severity in senior patients.

Platelet transfusion is a common therapy for bleeding

in hematology/oncology patients, post-cardiac surgery patients, trauma patients with acute hemorrhages and patients with thrombocytopenia and/or functionally abnormal platelets. Platelets have a short shelf life of up to 5–7 days. Over time in storage biochemical and functional changes occur in the platelets and therefore their storage medium (23). These changes, well-characterized as platelet storage lesions (PSL), include acidification of the storage medium and platelet activation among others (24). A recent study found that platelets from young donors exhibited better storage performances than those from older donors. Mitochondria functions were also found to be associated with PSL and post transfusion performances (25). Therefore, the discovery by Davizon-Castillo *et al.* that platelets from older individuals had increased mitochondria mass and metabolic reprogramming may partially help to explain the donor-to-donor variations in platelet transfusion efficacy and safety. As the authors have mentioned, the hyperreactive platelets with increased mitochondria mass have the potential of exacerbating inflammatory response by releasing higher amounts of mitochondrial DNA and other inflammatory mitochondrial components. Whether this will aggravate transfusion-related adverse events such as transfusion-associated sepsis and organ failure in patients who already have severe systemic inflammatory response; and on the other side, whether these hyperreactive platelets have higher efficacy in controlling bleeding, particularly for treatment of polytraumatic patients and those with massive bleeding, are worth further studies.

Acknowledgments

Funding: This work was supported in part by a grant-in-aid from the Heart and Stroke Foundation of Canada (Ontario); Canadian Institutes of Health Research (CIHR: MOP 119540, MOP 97918, MOP 68986 and MOP 119551), Canadian Institutes of Health Research Foundation grant (389035) and CIHR-Canadian Blood Services Partnership. ZYC and DK are recipients of the Fellowship from Department of Laboratory Medicine and Pathobiology, the University of Toronto.

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/aob-20-32). ZYC and DK report grants from Heart and Stroke Foundation of Canada, grants from Canadian Institutes of Health Research, grants from Canadian Institutes of Health Research Foundation grant, grants from CIHR-Canadian Blood Services Partnership, other from University of Toronto, during the conduct of the study; WM reports grants from Heart and Stroke Foundation of Canada, grants from Canadian Institutes of Health Research (CIHR), grants from Canadian Blood Services Centre for Innovation, other from CCOA Therapeutics Inc., during the conduct of the study; grants and personal fees from CCOA Therapeutics Inc., outside the submitted work; HN reports grants from Heart and Stroke Foundation of Canada, grants from Canadian Institutes of Health Research (CIHR), grants from Canadian Blood Services Centre for Innovation, other from CCOA Therapeutics Inc., during the conduct of the study; grants and personal fees from CCOA Therapeutics Inc., outside the submitted work; In addition, HN has a patent Anti-platelet integrin GPIIbIIIa monoclonal antibodies issued to CCOA Therapeutics Inc., and a patent Anti-platelet GPIbalpha monoclonal antibodies issued to CCOA Therapeutics Inc.. HN serves as an unpaid editorial board member of *The Annals of Blood* from June 2018 to June 2020.

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.

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doi: 10.21037/aob-20-32
Cite this article as: Ma W, Karakas D, Chen ZY, Ni H. Aging, chronic inflammation, and platelet hyperactivity. Ann Blood 2020;5:18.