part explain chronic inflammatory changes in old age.[7] He et al suggest another positive feedback loop, with increases in TNFα→IL-27Ra leading to greater immunosenescence and myeloid skewing, which itself leads to increased TNFα production from myeloid cells. But why would the chronic manifestation of these feedback mechanisms be mostly restricted to old age? Connecting the previous intestinal studies with the current research by He et al, one can surmise that TNFα/IL-27Ra–dependent changes in HSCs in old age indeed reflect a chronic manifestation, via intestinal permeability–facilitated entry of bacterial products, of the same antimicrobial responses that limit disease incidence throughout life. Humans and other animals simply did not evolve to live forever, and investments in tissue maintenance wane at ages at which the odds of contributing to future generations are historically low. Such investments include maintenance of our intestinal tract and its critical barrier function.

What on the surface seems to be contradictory, an important defense pathway that contributes to aging phenotypes later in life, actually makes a lot of sense. The concept of antagonistic pleiotropy was proposed >60 years ago by Williams[8]: genetically encoded phenotypes (or programs) that contribute to animal fitness in youth but which also promote aging phenotypes and reduced survival in old age are still favored by natural selection as long as, in balance, animal fitness (reproductive success) is increased. Because most animals in the wild do not survive to ages at which senescent phenotypes are evident, natural selection acts to maximize survival and reproductive success in youth even when these same programs contribute to our eventual demise in old age. Although the old mice studied by He et al were aged >2 years, mice rarely survive past 1 year in the wild, given high extrinsic hazards such as resource limitations, predation, disease, and cold. Only the luckiest, and rarest, wild mouse will die of "old age."

The million-dollar question of course is: is there anything we can do about these aging-related changes? Although the simple solution might seem to be to block inflammation in old age, we need to keep in mind this antagonistic pleiotropy, that inflammation is important for our defenses. In fact, while blocking IL-1β in people resulted in decreases in cancers and heart disease, overall survival was not improved due to increases in deaths by infections.[9] Still, a more mechanistic and detailed understanding of the pathways controlling functional decline in old age, as provided for HSCs by He et al, could lead to more nuanced and targeted strategies to mitigate these declines without overly compromising the evolved functions of the pathways underlying these aging-associated perturbations.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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DOI 10.1182/blood.2020006070

THROMBOSIS AND HEMOSTASIS

Comment on Garnier et al, page 247

Sickle particulars of microparticles

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According to new data by Garnier et al in this issue of *Blood*, microparticles derived from red blood cells provoke an endothelial cell proinflammatory phenotype that is mitigated by hydroxyurea.[1]

Sickle cell disease, caused by a simple single nucleotide mutation, continues to display humbling complexity in its downstream pathophysiology of intravascular hemolysis and inflammatory endothelial response. Polymerization of sickle hemoglobin promotes oxidative stress in red blood cells, with wide-ranging damage to the red blood cell plasma membrane, cytoskeleton, membrane channels, cytoplasmic metabolites, and antioxidant self-repair mechanisms. Among the subpopulations of red blood cells in sickle cell disease, some expose phosphatidylserine (PS), marking senescent red blood cells for deletion by reticuloendothelial macrophages; this adaptive pathway is called extravascular hemolysis.

The extensive impairment of red blood cell homeostasis in sickle cell disease leads to metabolically and mechanically fragile red blood cells. These extensively damaged red blood cells can lyse in the circulation, termed intravascular hemolysis. This more pathologic form of hemolysis releases red blood cell contents into plasma, where it is toxic to endothelial cell function. Cell-free hemoglobin, arginase-1, and red blood cell metabolites interfere with nitric oxide bioavailability and contribute to vasculopathy in patients with sickle cell disease.[2] In addition, cell-free hemoglobin and heme serve as danger signals, priming the innate immune system.

Damaged, fragile red blood cells also generate submicron fragments called

microparticles. Red blood cell microparticles are detected in large numbers in plasma from patients with sickle cell disease, malaria, hereditary spherocytosis, and glucose-6-phosphate dehydrogenase deficiency.[3] The shared pathobiology includes weakening of the connections of the lipid bilayer of the cell membrane to the underlying cytoskeletal proteins, involving phosphorylation of band 3 and oxidation of hemoglobin. The unanchored membrane can bud off from the damaged red blood cell, carrying along exposed PS, small amounts of hemoglobin, and surface CD41 that marks its erythrocyte origin. Initial characterizations showed the effects of red blood cell microparticles on coagulation and thrombosis.

Extracellular vesicles (a more general term) are highly abundant in the plasma of patients with sickle cell disease, generated from red blood cells, platelets, leukocytes, and even endothelial cells.[4] Extracellular vesicles derived from platelets from humans or mice with sickle cell disease carry a payload of interleukin-1β and caspase-1 that promotes platelet–neutrophil aggregation in the pulmonary blood vessels.[5] Microparticles derived from red blood cells have several pathophysiologically relevant properties in sickle cell disease. Hemoglobin in red blood cell–derived microparticles can scavenge nitric oxide,[6] suggesting that microparticles are part of the spectrum of hemolysis-linked mechanisms that interfere with vasomotor function and endothelial health.[2] The uniquely oxidative environment inside the sickle erythrocyte yields numerous redox forms of oxidized hemoglobin (including the ferryl state) that are abundant in microparticles.[7] These oxidation reactions seem to promote the release of microparticles and also to progress in the microparticles themselves. Oxidized hemoglobin is prone to release free heme, which is strongly implicated in sickle cell disease pathophysiology. Red blood cell microparticles can transfer heme to endothelial cells, inducing oxidative stress and apoptosis in cultured endothelial cells and renal vaso-occlusion in mice with sickle cell disease.[8] They have been found adherent to capillary endothelium in the glomeruli of patients with sickle cell disease and albuminuria. Red blood cell microparticles can activate complement, which likely plays a role in patients with sickle cell disease and in delayed hemolytic transfusion reactions[9]; this topic warrants deeper investigation. All these properties of red blood cell microparticles contribute to a proinflammatory state that promotes adhesiveness of circulating cells to each other and to endothelium.[10]

In addition, Garnier et al provide evidence that red blood cell–derived microparticles play a key role in the pathophysiology of sickle cell disease by triggering a proinflammatory phenotype of endothelial cells. They purified microparticles from sickle cell plasma of red blood cell origin and separate microparticles of platelet origin. Sickle red blood cell–derived microparticles adhered to cultured endothelial cells, especially those microparticles with the highest density of PS. This action induced endothelial expression of ICAM-1, making them adherent to neutrophils. This finding is particularly relevant, because growing evidence suggests that inflammation-stimulated neutrophil adhesion underlies the pivotal event in sickle cell vaso-occlusion.

Garnier et al show that hydroxyurea treatment of patients with sickle cell disease definitively reduces hemolysis and generation of red blood cell microparticles. They and several other groups have previously observed that circulating red blood cell microparticles are less abundant in plasma of patients with sickle cell disease being treated with hydroxyurea compared with patients not taking hydroxyurea. The interpretation of results from such cross-sectional studies suffers from inherent confounding by indication. However, the researchers evaluated red blood cell microparticles in paired plasma samples from the same patients before and after 1 or 2 years of hydroxyurea therapy. This approach allowed them to clearly show that hydroxyurea reduces circulating red blood cell microparticle levels and proadhesive activity. This result more firmly documents yet another hemolysis-linked pressure point in sickle cell pathophysiology that is improved by hydroxyurea. It adds to many other converging lines of evidence further supporting the use of hydroxyurea in sickle cell disease. In addition, DeBaun[11] proposes extension of hydroxyurea use for patients with cerebral vasculopathy and stroke risk with impaired access to monthly transfusion therapy.

Garnier et al also document that neutrophil adhesion to endothelial cells under the influence of red blood cell microparticles can be inhibited by either the PS-blocker protein annexin V or a blocking antibody against ICAM-1. These potential therapeutic targets in sickle cell disease are not new, but they are supported by these new data. The particulars of microparticles in sickle cell disease highlight an additional facet of hemolysis in the pathobiology of sickle cell disease.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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DOI 10.1182/blood.2020006303