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PERSPECTIVE

New insight into the causes, consequences, and correction of hematopoietic stem cell aging

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Aging of hematopoietic stem cells (HSCs) is characterized by lineage bias, increased clonal expansion, and functional decrease. At the molecular level, aged HSCs typically display metabolic dysregulation, upregulation of inflammatory pathways, and downregulation of DNA repair pathways. Cellular aging of HSCs, driven by cell-intrinsic and cell-extrinsic factors, causes a predisposition to anemia, adaptive immune compromise, myelodys, plasia, and malignancy. Most hematologic diseases are strongly associated with age. But what is the biological foundation for decreased fitness with age? And are there therapeutic windows to resolve age-related hematopoietic decline? These questions were the focus of the International Society for Experimental Hematology (ISEH) New Investigator Committee Fall 2022 Webinar. This review touches on the latest insights from two leading laboratories into inflammatory- and niche-driven stem cell aging and includes speculation on strategies to prevent or correct age-related decline in HSC function.

HIGHLIGHTS

- Low-grade inflammation in early to mid-life accelerates mouse hematopoietic aging
- The decline of niche factor insulin-like growth factor 1 drives the onset of hematopoietic aging
- Middle age provides a window of opportunity for interventional strategies

The human lifespan has doubled over the past 200 years reflecting significant progress in sanitation and medicine [1]. However, this increase in lifespan presents us with new socioeconomic and medical challenges. Somatic stem cells, including hematopoietic stem cell (HSCs), are tasked with maintaining, repairing, and replenishing tissues throughout life. Thus, their lifespan, unlike the bulk of cells that make up the human body, equals or exceeds the lifespan of the organism [2,3]. This responsibility for regeneration in combination with their uniquely long lifespan confers vulnerability in the system: age-related decline of stem cell fitness directly impacts organismal quality of life and risk of cancer development [3]. Therefore, there is significant interest in understanding and therapeutically targeting the fundamental processes underlying biological stem cell aging.

Murine HSC aging, largely defined by studies of the inbred C57BL/6 mouse strain, is characterized by increased markers of DNA damage, mitochondrial dysfunction, increased reactive oxygen species, loss of polarity, and upregulation of inflammatory signals [4,5] largely consistent with the hallmarks of aging [6,7]. Consequently, the phenotypic HSC pool expands numerically and becomes myeloid-biased, regeneration-deficient, and increasingly clonal [8–10]. Human HSC aging remains less explored but shows evolutionarily conserved changes including phenotypic stem cell pool expansion, myeloid bias, loss of polarity, and functional alterations [11,12]. Direct hematologic consequences of HSC aging include the onset of anemia and (pre)malignancy. More systemically, hematopoietic aging increases the risk of immune dysfunction, thromboses, chronic inflammation, and age-related clonal hematopoiesis (CH), which in turn is linked to cardiovascular complications, type 2 diabetes, and atherosclerosis [13]. Chronic inflammation is a well-documented contributor to HSC aging. The term "inflammaging" encompasses the processes in which inflammation both drives and is driven by HSC aging (through cell-intrinsic and cell-extrinsic mechanisms [14–18]). However, thus far it was not known whether the inflammatory challenge in early- to mid-life impacts on the process of physiologic aging. In Bogeska et al.'s work [19], an important insight is gained into how inflammatory challenge in early life irreversibly

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impacts HSC function in a dose-dependent and aging-resembling manner.

Although Bogeska et al. [19], investigated challenge in early- to mid-life, Young et al. [20] investigated if mid-life provides a therapeutic window for correction or prevention of HSC aging. They showed that HSC aging is initiated in middle life and identified a single niche factor, insulin-like growth factor 1 (IGF1), as critical to the onset of hematopoietic aging. This review will place the findings of Bogeska et al. [19] and Young et al. [20] in the context of what is known and what is still unknown about the causes, consequences, and correction of HSC aging.

CHRONIC INFLAMMATION ACCELERATES HSC AGING

The concept that aging drives the development of many chronic inflammatory conditions such as metabolic syndrome and atherosclerosis is a well-recognized phenomenon [21,22]. On the contrary, whether inflammatory exposure during early- to mid-life accelerates the aging process is much less understood. Importantly, many earlier studies have demonstrated that acute inflammation can lead to dramatic suppression of HSC self-renewal capacity and altered lineage output [14–18,22–24]. However, whether chronic inflammation can elicit sustained long-term decline in HSC functionality is not known. During the ISEH winter webinar, Dr. Mick Milsom presented a recently published study from his laboratory investigating the effect of repetitive low-grade inflammatory challenges on HSC aging [19] (Figure 1).

A key experimental model of the study is to treat mice repeatedly with polyinosinic:polycytidylic acid (pI:pC), a toll-like receptor 3 (TLR3) agonist that mimics viral infection and elicits a type 1 interferon response. It is known that various short-term inflammatory challenges induce transient and reversible effects on the hematopoietic system, whereby long-term HSCs (LT-HSCs) enter the cell cycle but return to the same level of dormancy after a few days [24,25]. Here, escalated doses of pI:pC (one to three blocks of treatment; eight injections per block) were used and analysis was conducted after a 4-week recovery period to study the long-term effect of inflammation. No changes in cellularity and cell-type composition persisted in the peripheral blood (PB) and bone marrow (BM) of these mice. Despite that, Dr. Milsom's team demonstrated substantial long-term impairment of HSC function via a series of in vitro and in vivo assays [19].

First, an in vitro single-cell liquid culture assay was used to compare the proliferation and differentiation of pI:pC-treated HSCs with those isolated from phosphate buffered saline (PBS)-treated control animals. They observed a marked reduction in the number of proliferative HSC clones, as well as the overall number of cells produced from individual HSCs. Consistently, competitive transplantation of HSCs treated with one, two, or three blocks of pI:pC revealed a dose-dependent reduction in reconstitution capacity, indicating progressive loss of functional HSCs. Strikingly, even after a longer recovery period of 5, 10, or 20 weeks, functional HSCs failed to regenerate and return to a homeostatic level. To further understand whether the observed functional defects were intrinsic to HSCs, the authors performed reverse transplantation experiments, whereby excess numbers of HSCs were transplanted into unconditioned recipient mice injected with PBS, or challenged with three blocks of pI:pC, followed by 5, 10, or 20 weeks recovery. Importantly, robust donor reconstitution was observed in pI:pC-treated hosts, especially those with longer recovery after stimulation, suggesting sustained

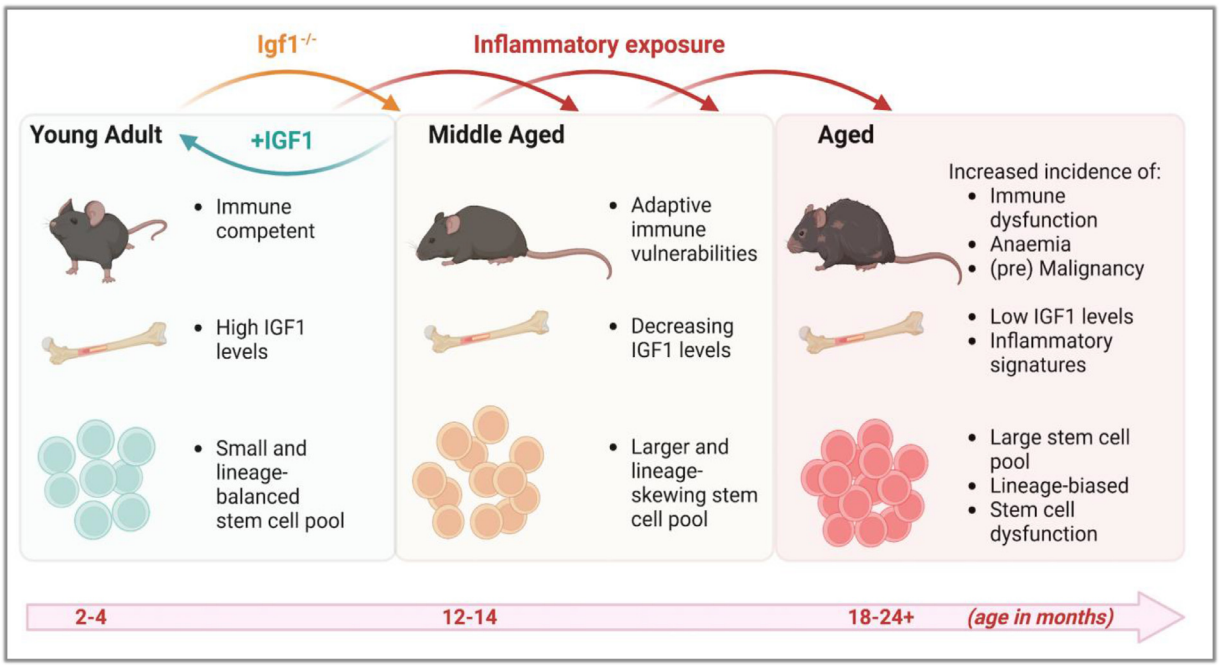

Figure 1 Graphical summary of new insight generated by the Milsom and Trowbridge laboratories. Briefly, Young et al. [20] show that decreasing IGF1 levels during middle age are a central player in the onset of HSC agiing. Furthermore, Bogeska et al. [19] highlight that repeated low-grade inflammatory challenges in early life to middle age cause stable changes in HSCs strongly resembling premature aging. HSC=hematopoietic stem cell; IGF=insulin-like growth factor 1.

impairment of HSCs in these recipient mice. Finally, to recapitulate a more physiologically relevant scenario, monthly *Mycobacterium avium* infection (3 ×) was used to challenge the mice [26], followed by an extensive recovery of 4 or 20 weeks. Similar to the sterile inflammation from pI:pC treatment, HSC function was compromised and failed to recover after repetitive infection with *M. avium*. Taken together, a series of rigorous experiments were performed in this study to demonstrate that recurrent inflammatory exposures have a cumulative effect and lead to substantial and durable depletion of functional HSCs [19].

To gain insight into whether the observed functional impairment induced by chronic inflammation accelerates HSC aging, the mice were again treated repeatedly with pI:pC (3 × 8 injections) and analyzed after 12 months. Several typical aging phenotypes were observed, which included the following: (1) age-associated accumulation of HSCs, (2) sustained defect in HSC function, (3) profound myeloid-biased differentiation, and (4) a marked reduction in HSC clonal diversity. At the molecular level, pI:pC-treated HSCs exhibited profound loss of polarity of the H4K16ac mark, a previously described phenomenon in aged HSCs [27]. In addition, DNA methylome clock analysis was performed to predict the biological ages of HSCs, based on the noti-on that cells progressively gain methylation at certain CpG loci during aging [28,29]. Strikingly, the methylome of middle-aged HSCs exposed to pI:pC treatment was highly similar to those from aged control mice. Importantly, 8-week-old mice exposed to pI:pC that were analyzed at 24 months of age presented with cytopenias, BM hypocellularity, and increased BM adipocytes, validating that early-life exposure to inflammatory challenge indeed causes compromised hematopoiesis with age. Notably, these are common features of nonmalignant aged hematopoiesis in humans who do not spontaneously develop in aged laboratory mice, suggesting that the lack of environmental stimuli in mice housed under highly controlled conditions might be a factor that restricts the attainment of some features of normal human aging. Collectively, these results convincingly demonstrate that HSCs exposed to inflammation in early life manifest hallmarks of accelerated aging at the functional and molecular level, which cannot be explained by the natural progression of aging alone [19].

The authors next used the Scl-tTA;H2B-GFP mouse model [30] to capture HSCs that underwent in vivo cell division in response to the inflammatory stimulus, in this case using only a single block of 8 pI:pC injections [19]. Importantly, although this treatment regimen is sufficient to drive some of the label-retaining HSCs into the cycle, corresponding with a significantly increased output of HSCs to the production of most major blood lineages [31], a reserve of dormant label-retaining cells (LRCs) can still be identified. In this setting, LRCs maintained their functional potency in vivo and in vitro, whereas the non-LRCs that mounted a proliferative response to inflammation lost their functional potency. This demonstrates that in vivo HSC self-renewal divisions are very rare or nonexistent during the proliferative response to this inflammatory stimulus, and suggests that HSCs that maintain a long-term dormant status in the face of lifelong inflammatory episodes will be protected from the inflammation-induced decline in functionality. Taken together, this new study from Dr. Milsom's group [19] has established the concept that repetitive low-grade inflammatory challenges during early- to mid-life lead to progressive decline in the number of functional HSCs, a process that does not naturally recover and results in the acceleration of HSC aging. These results have important implications for further understanding the phenomenon of HSC exhaustion during aging and provides opportunities for developing potential strategies to increase HSC regeneration.

WHEN AND HOW DOES HSC FUNCTION DECLINE WITH AGE?

HSCs functionally decline with age, losing self-renewal and regenerative potential (reviewed by [4,32]). Many of the key studies determining the hallmarks of HSC aging have compared HSCs from young mice (2–6 months of age) with those from old mice (18–24 months) – equivalent to comparing humans aged 20–30 years with those aged 56 to 69 years [32]. However, the binary comparison of old and young HSCs provides an incomplete understanding of HSC aging dynamics. Cross-sectional studies incorporating multiple ages are required to address when aging-associated HSC phenotypes emerge, whether there is an order in which they emerge, how they progress over time, and when they become irreversible. Recent studies from Dr. Jennifer Trowbridge's laboratory have highlighted middle age (10–14 months) as a distinct stage of HSC life history where the first emergence of hallmarks of HSC aging can be observed including myeloid-biased hematopoiesis, expansion of the phenotypic HSC population, and accumulation of DNA damage markers [20,33].

Factors controlling the emergence of HSC aging phenotypes can be investigated in middle-aged mice. Reciprocal BM transplant experiments, in which middle-aged HSCs are transplanted into young recipients and vice versa, can determine the contribution of HSC-intrinsic and -extrinsic factors to the early stages of HSC aging. Transplantation of middle-aged HSCs into the BM microenvironment of young mice reduced their aging-associated myeloid-lineage bias. Conversely, the middle-aged BM microenvironment increased the myeloid output of young HSCs [20]. Underlying these changes, middle-aged HSCs downregulated aging-associated myeloid- and immune-associated gene signatures after being returned to the young BM environment. HSC-extrinsic factors in the BM, therefore, make a significant contribution to the age-associated myeloid priming of HSCs, and this priming can be at least partially reversed in middle age by exposure to a young BM microenvironment.

Transcriptional analysis of isolated mouse LT-HSCs showed that young, middle-aged, and old HSCs have distinct gene expression profiles, with young HSCs being more distinct from the other two populations. Gene signatures particularly enriched during middle age include decreased metabolism and mTORC1 signaling [20]. Similarly, single-cell RNA-seq analysis of young and middle-aged HSCs revealed dysregulation of gene signatures associated with mitochondria, oxidative phosphorylation, and metabolism [33]. The mitochondria of middle-aged HSCs have dysregulated, fragmented morphology, and the decreasing mitochondrial membrane potential that occurs as the age of HSCs is strongly associated with their functional decline [20,34].

Upstream regulator analysis predicted that transcriptional changes in middle-aged HSCs may be caused by increased IGF2 and decreased IGF1, neuregulin 1 (NRG1), transforming growth factor-β (TGF-β1), and epidermal growth factor (EGF) in the middle-aged BM microenvironment [20]. IGF1 and IGF2 are expressed by nonhematopoietic cells in the BM, and the amount of IGF1, but not IGF2, decreases in the BM fluid as mice reach middle age [20,35]. The

transplantation of HSCs into IGF1-deficient mice, or deletion of the IGF1 receptor on HSCs, each prematurely induce myeloid-biased hematopoiesis. Together these data show that reduced IGF1 expression in the aging BM environment contributes to the functional decline of HSCs.

To determine which BM cells are contributing to declined IGF1 levels during aging, single-cell RNA-seq of hematopoietic and stromal cells isolated from young and middle-aged BM was performed. The cellular composition of the stromal compartment became more variable between individual mice with age (unpublished data). There was also a trend toward an increased proportion of fibroblasts and adipomesenchymal stem and progenitor cells (MSPCs), and a decreased proportion of endothelial cells. *Igf1* expression decreased in MSPCs, fibroblast, and osteoblasts at middle age. Next, IGF1 was deleted from BM stromal cells by Cre recombinase expression under the control of the *Nestin, Prx1*, or *Lepr* promoters. In all three mouse models, this stroma-specific IGF1-deficiency was sufficient to induce myeloid-biased hematopoiesis (unpublished data).

To determine whether IGF1 could rescue the declining function of middle-aged HSCs, isolated LT-HSCs were treated with IGF1 before transplantation. Sixteen hours of IGF1 treatment was sufficient to restore mitochondrial activity and reverse aging-associated myeloid bias [20]. IGF1 treatment altered HSC chromatin, reverting it to a state similar to young HSCs. Promoters associated with mTORC1 signaling, metabolism, cell cycle, and IL-2/STAT5 signaling were more accessible, whereas promoters associated with apoptosis, hypoxia, IFN-γ, and NF-κB were less [20].

The study of middle-aged HSCs has revealed that the phenotypes observed in very old HSCs begin to manifest much earlier in life. HSC-extrinsic factors in the microenvironment make a substantial contribution to the onset of these aging phenotypes during middle age. Importantly, a young BM microenvironment, or even transient exposure to a single growth factor from the young BM, can rejuvenate middle-aged HSCs, at least partially rescuing multiple hallmarks of aging. In contrast, recent work has shown that reprogramming HSCs from very old mice into a younger, more functional state through exposure to young blood or niches is extremely challenging [36,37]. This suggests that middle age may offer a window of opportunity for such intervention, in which the functional decline of HSCs can be decelerated, or even reversed. Further research on HSCs at multiple ages will deepen our understanding of the life histories of HSCs, and whether it is possible to slow, halt, or undo their decline.

CONCLUSION AND PERSPECTIVE

The studies from Dr. Milsom and Dr. Trowbridge's laboratories shed new light on the underlying causes and sequential trajectories of HSC aging and highlight new windows in which aging can be initiated or halted in its tracks. Rejuvenation of HSC aging remains a challenging but sought-after goal. These studies show that niche factor depletion or inflammatory stimulation in early life causes stable age-resembling changes in HSCs that do not recover without intervention. This suggests that middle age might provide a window for prophylactic anti-inflammatory treatment or other interventional strategies. Attempts to directly or indirectly target the mitochondrial metabolism of middle-aged or aged murine HSC have yielded promising results in alleviating aspects of HSC aging [20,34,38]. However, it is not fully understood if and how these changes in mitochondrial biology are causative of, or simply correlate with, the correction of other hallmarks of aging. Another possible avenue to explore involves exploiting HSC heterogeneity, through recruitment or expansion of the rare residual functional HSCs or selective depletion of (clonally expanded) dysfunctional HSCs. It is worth noting that biological sex−dependent differences in the onset and magnitude of the hematopoietic aging phenotype warrant further scrutiny [39], as well as how these relate to human biology. Although it has been suggested that hematopoietic aging is an evolutionarily conserved process [11], the extent to which the inflammatory- and niche-driven HSC aging phenotype in mice represents all aspects of physiologic HSC aging in humans, and across diverse mouse strains, needs to be further addressed. This includes the increased risk of malignant transformation and the extent to which treatments reverse any or all aspects of HSC aging. Answering these questions will reveal fundamental aspects of stem cell aging and will help refine interventional approaches.

Conflict of Interest Disclosure

The authors do not have any conflicts of interest to declare in relation to this work

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