



Aging of the hematopoietic system

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Purpose of review

Aging of the hematopoietic system is associated with myeloid malignancies, anemia and immune dysfunction. As hematopoietic stem cells (HSCs) generate all cells of the hematopoietic system, age-associated changes in HSCs may underlie many features of the aged hematopoietic system. Recent findings on age-associated changes in HSCs are reviewed here.

Recent findings

Aged HSCs are myeloid biased, have acquired DNA damage and are functionally compromised. However, overall function of the HSC compartment is well maintained through age-associated expansion of HSCs. Many age-related changes in the hematopoietic system, in particular the clonal myeloid bias of HSCs and the decrease in B and T-cell development, in fact begin during development. Furthermore, HSCs possess specific protective mechanisms aimed at maintaining their number, even at the expense of accumulating damaged cells.

Summary

We argue that age-related changes in HSCs and in the hematopoietic system may not entirely be due to a degenerative aging process, but are the result of developmental and stem cell-protective mechanisms aimed at maximizing fitness during reproductive life. These mechanisms may be disadvantageous later in life as damaged HSCs accumulate and establishment of responses to neoantigens becomes compromised because of the reduced generation of naive T and B cells.

Keywords

aging, development, hematopoietic stem cells

INTRODUCTION

Similar to most organs and tissues, the hematopoietic system shows evidence of aging, which is associated with increased incidence of myeloid malignancies, myelodysplasia, myeloproliferative neoplasms, chronic anemia and multifactorial immune dysfunction [1–3]. Furthermore, donor age is a major negative prognostic factor in the outcome of allogeneic bone marrow transplantation [4]. As stem cells assure tissue maintenance, it is logical to infer that hematopoietic stem cell (HSC) dysfunction underlies aging of the hematopoietic system.

THE FUNCTIONAL PHENOTYPE OF AGED HEMATOPOIETIC STEM CELLS

In mice, aging is associated with expansion of the HSC compartment [5–8], such that old bone marrow can even outcompete equal amounts of young bone marrow [9]. Furthermore, hematopoietic reconstitution from aged HSCs is myeloid-biased compared with reconstitution by young HSCs [8,10–12]. The most rigorous functional analysis

of aged HSCs was reported by Dykstra *et al.* [7], who showed that aged HSCs are, on a per cell basis, inferior to young HSCs in their capacity to reconstitute lethally irradiated recipients and to self-renew, as assayed in serial transplantation studies. Furthermore, homing [13] and in-vitro proliferation in stromal cocultures were reduced. Reconstitution of the HSC compartment by aged HSCs was normal [14[†]]. Intravital microscopy suggested that early progenitors from aged mice localize further from the presumed osteoblastic niche in the bone marrow than cells from young mice, and display more dynamic changes in cell shape [15]. Furthermore, upon adhesion to fibronectin, early progenitors from aged mice show reduced polarization of the

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KEY POINTS

- Aging of HSCs is associated with expansion of stem cell number, decreased function of individual stem cells, increased myeloid bias, decreased polarity, and increased DNA damage. The overall function of the HSC compartment is maintained, however.
- These changes are predominantly intrinsic.
- Expansion of the stem cell compartment, increased myeloid bias, and a decrease in lymphopoiesis start early in life, and may therefore be developmentally programmed.
- Hematopoietic stem cells possess intrinsic stem cell-protective mechanisms that preserve the function of the stem cell compartment as a whole, but may be accompanied by accumulation of damaged stem cells.
- Both developmentally programmed changes and stem cell-protective mechanisms contribute to age-related changes in HSC function and in the hematopoietic system.

microtubule cytoskeleton compared with cells from young mice [15]. Distinct niche interactions are also suggested by the observation that progenitor cells from old mice are mobilized more efficiently [16]. Together, most available data suggest intrinsically compromised function of individual aged HSCs, but maintenance of overall function of HSCs as a population. Several lymphoid precursors, such as common lymphoid progenitors (CLPs) and, in particular, pre-B cells are depleted in the bone marrow of old mice [3]. Lymphopoiesis is not only affected by HSC-intrinsic changes, however. The age-associated decline in pre-B cells appears mostly caused by the aged microenvironment [17], whereas T-cell development is severely curtailed by thymic involution [18]. Lymphocyte number is maintained, however, because homeostatic proliferation fills the void with antigen-experienced cells (memory T cells; marginal zone, B1 and memory B cells) [2,3,19].

Similar to mice, the frequency of human HSCs increases with age, and the HSC compartment shows a myeloid bias at the population level when assayed by xenotransplantation in immunodeficient mice [20].

CONSEQUENCES OF AGING OF HEMATOPOIETIC STEM CELLS

The negative impact of donor age on the outcome of allogeneic bone marrow transplantation appears mostly due to higher incidence and severity of graft-versus-host disease, and not to an engraftment

defect [4]. It is tempting to speculate, however, that the increased incidence of myeloid malignancies in the elderly can be traced back to the myeloid bias of HSCs. HSCs, the only persistent lineage of hematopoietic cells, serve as a repository for accumulating DNA mutations that can confer abnormal self-renewal properties to downstream progenitor cells, leading to leukemia [1]. Furthermore, the lower incidence of lymphoid malignancies originating from developing B and T cells in the aged compared with the young [21] may be caused by impaired lymphopoiesis. The fact that the most prevalent hematological malignancy in the elderly is chronic lymphocytic leukemia (CLL) [22] is often overlooked in this context, however. CLL is a malignant, clonal B-cell expansion with the phenotype of antigen-experienced B cells. Although originally thought to be driven by chronic stimulation by as yet undefined antigens [22], recent evidence suggests that cell autonomous signaling through the B-cell receptor (BCR) in response to internal BCR epitopes drives this malignancy [23^{*}]. Nevertheless, xenotransplantation studies in immunodeficient mice have shown that the propensity to generate oligoclonal or monoclonal B-cell development originates in HSCs of CLL patients [24]. In this context, it is also interesting to note that in mice with a transgenic BCR, B cells with a phenotype of antigen-experienced cells and bearing the endogenous BCR increase with age [25]. Transplantation of purified HSCs from old mice to young mice transferred these altered B-cell specificities. Furthermore, transplantation of small numbers of young HSCs or treating recipients with anti-interleukin-7 antibodies, two approaches that compromise the B-cell regenerative capacity of the hematopoietic system, also skewed the B-cell population toward antigen-experienced cells expressing the endogenous BCR [26]. It is therefore plausible to hypothesize that aging of HSCs, and therefore their reduced lymphoid potential and overall functional compromise, may contribute to a skewed B-cell repertoire and to a propensity to develop CLL or its precursors.

MOLECULAR PHENOTYPE OF AGED HEMATOPOIETIC STEM CELLS

Genome-wide expression studies comparing HSCs from young and old mice showed that in aged HSCs, expression of genes involved in myeloid development was increased whereas expression of genes involved in lymphopoiesis was decreased, a finding consistent with their myeloid bias [8]. Furthermore, gene sets associated with inflammation were increased in aged HSCs [8,27]. Young and aged HSCs differ in epigenetic regulation. Aged HSCs show

general hypermethylation [14[■]]. This was surprising, as aging in most tissues is associated with hypomethylation [28]. Interestingly, however, differentially methylated regions were enriched in genes expressed in downstream lineages and not, or less so, in HSCs themselves, and included genes involved in erythropoiesis and lymphopoiesis. Furthermore, a fraction of these genes are targets of the polycomb repressor complex (PRC)2, and expression of several PRC2 components was slightly reduced in aged HSCs [14[■],29]. Downregulation of genes involved in transcription and chromatin regulation, as well as concomitant misexpression of lymphoid transcripts such as Ig κ germline transcripts, was observed in another study [27]. A fraction of HSCs in aged mice also display lower histone H4 lysine acetylation (AcH4K16) [30[■]], providing further evidence for changes in epigenetic regulation. Aged HSCs express higher levels of the RhoGTPase cdc42, and this increased expression was associated with the decreased polarity and decreased AcH4K16 observed in aged HSCs compared with young HSCs [30[■]]. Finally, an age-associated increase in DNA damage as measured by γ H2AX foci has been observed in HSCs, suggesting a role for accumulated DNA damage [31].

THE ROLE OF PROLIFERATIVE HISTORY

Proliferation of most normal cells ultimately leads to senescence. Adult HSCs are remarkably quiescent, however, though they can reversibly enter and exit cell cycle, even in steady state [32,33,34[■]]. Given their relative quiescence, the expansion of the normally cycling aged HSC compartment is unlikely to be explained by cellular senescence. Furthermore, despite earlier suggestions [35], the p16INK4a locus, a prime regulator of senescence, remains epigenetically repressed in aged HSCs [29].

Proliferation is accompanied by telomere shortening. Humans have shorter telomeres than inbred mice, and progressive telomere shortening is observed in the peripheral blood, a proxy of telomere shortening in HSCs [36,37]. Although genetic telomere dysfunction in humans causes dyskeratosis congenita, a disease with a high incidence of bone marrow failure, and although acquired severe aplastic anemia is associated with short telomeres [36,37], the role of telomere shortening in physiological aging of human HSCs has not been determined [38]. HSCs fail in late generation telomerase-deficient mice [39]. However, maintenance of mouse HSCs is not dependent on telomeres, as increasing telomerase activity in HSCs through overexpression of telomerase does not rescue HSCs

from exhaustion induced by serial transplantation [40]. Other mechanisms must therefore be invoked to explain HSC exhaustion after serial transplantation.

Nevertheless, forced proliferation of mouse HSCs by serial transplantation [7], transplantation of very low numbers of HSCs [14[■]] or repeated administration of 5-fluorouracil [14[■]] induced changes similar to those observed in physiological aging, namely lower reconstitution capacity and myeloid bias. These findings may suggest a contribution of proliferative history to age-related changes in HSCs. It should be noted, however, that in serial transplantation studies, the chronological age of young HSCs that underwent three rounds of serial transplantation (approximately 20 months) was similar to what is considered 'aged' in C57BL/6 mice [7]. It is therefore unclear to what extent functional changes in the HSC compartment in serial transplantation studies are caused by chronological aging, or by forced proliferation, or by a combination of both. Furthermore, forced proliferation of HSCs may differ from physiological aging. Repeated 5-fluorouracil administration, although inducing a functional phenotype similar to that of aged HSCs, was associated with global hypomethylation. On the other hand, physiological aging of HSCs was associated with global hypermethylation, although a fraction of differentially methylated regions was shared between physiologically aged HSCs and HSCs subjected to forced proliferation [14[■]]. Thus, although proliferative history may contribute to physiological HSC aging in mice, the latter is not fully modeled by forced cycling and expansion of HSCs.

THE ROLE OF INFLAMMATION

Aging is characterized by a state of low-level inflammation, that may both cause and perpetuate the aging process [41]. The aging hematopoietic system may itself be a source of inflammatory mediators. Thymic involution leads to a decreased pool of naive T cells. Through peripheral expansion, memory cells fill the void in the T-cell pool in aged individuals [19,42]. This accumulation of senescent memory cells, by virtue of their constitutive production of inflammatory cytokines, has been linked to the general state of low-level inflammation and frailty that characterizes aging ('inflammaging') [42]. A common feature of genome-wide expression studies of aged HSCs is the preponderance of genes involved in inflammation among upregulated genes [8,27]. Furthermore, recent work has shown that HSCs participate in systemic inflammatory responses [43] and respond directly to inflammatory cytokines

such as interferons [44,45], and that deletion of mechanisms that dampen the effect of interferons on HSCs leads to enhanced cycling and exhaustion [46,47]. Inflammation may therefore contribute to HSC aging. However, it is also possible that the increased inflammatory gene expression in HSCs is a reflection of the inflammatory environment in aged individuals, and not necessarily causally related to age-related changes in HSCs.

THE ROLE OF STEM CELL-PROTECTIVE MECHANISMS

Continuous exposure to extrinsic (such as low-dose environmental irradiation) or intrinsic [such as reactive oxygen species (ROS) generated by cellular metabolism] stressors can lead to stem cell dysfunction, and affect tissue integrity. HSCs are endowed with specific protective mechanisms, however. Quiescence likely protects from senescence [34[■]]. HSCs are exquisitely sensitive to ROS, predominantly produced by mitochondrial respiration [48]. Mechanisms that reduce ROS production and/or increase ROS scavenging in HSCs include Atm [49], Foxo transcription factors [50] and the reliance on glycolysis for ATP production [51]. HSCs have an increased capacity of autophagy in response to stress compared with progenitor cells, which undergo apoptosis in the same conditions [52[■]]. Interestingly, autophagy and ROS production and scavenging are regulated by members of the Foxo family [50,52[■]], which are involved in lifespan regulation in *Caenorhabditis elegans* [53], suggesting that HSCs have adopted organismal maintenance mechanisms of lower organisms. HSCs are more radioresistant than myeloid progenitors, among others, because of robust induction of DNA damage checkpoints and high expression of prosurvival genes and p21. Because of their quiescent nature, HSCs use the error-prone nonhomologous end-joining (NHEJ) pathway, whereas cycling progenitors use homologous recombination to repair irradiation-induced DNA double-strand breaks. However, even after HSCs are induced to cycle, and partially rewire their DNA repair toward homologous recombination, they do remain more radioresistant than progenitors [54]. The use of the error-prone NHEJ leads to persistent DNA damage in HSCs, and may cause malignancy, although the latter has not been formally demonstrated [54]. The gene *Batf* is induced upon DNA damage, inhibits self-renewal, and drives the cells toward lymphoid differentiation. As lymphoid-biased HSCs appear more sensitive to the effect of *Batf*, this mechanism may contribute to depletion of lymphoid biased HSCs and relative maintenance of myeloid biased HSCs

[55]. Taken together, most available evidence suggests that the HSC compartment is maintained through attempted repair at the cost of retaining damaged cells rather than replacing these by enhanced self-renewal.

Aging might be caused by failure of these protective mechanisms. However, there is little evidence that stem cell-protective mechanisms overtly fail in aging HSCs. Increased autophagy is maintained in aged HSCs [52[■]]. Genome-wide expression studies did not reveal major changes in genes essential for maintenance of quiescence in HSCs [8,27], and HSC cycling does not increase with age. Although genetic deletion of stem cell-specific protective mechanisms results in stem cell dysfunction, these phenotypes may represent frank stem cell failure rather than physiological aging [34[■]]. For example, knockout mice in which components of DNA repair mechanisms were deleted show mostly defects in downstream progenitor cells in steady state, and HSC function is only compromised after transplantation of limiting numbers of HSCs [31]. Myeloid/lymphoid skewing is not observed in these knockout mouse models [56]. Thus, failure of mechanisms that protect HSCs from various forms of stress is unlikely to explain age-related changes in HSCs.

An alternative hypothesis is that age-related changes in HSCs may at least in part be a result of the very mechanisms aimed at maintaining function of the overall HSC compartment. As mentioned before, the HSC compartment is geared toward maintenance through prevention of damage by ROS, and through attempted repair rather than replacing HSCs by enhanced self-renewal. This mode of maintenance of the HSC compartment may have evolved because of the apparently extreme sensitivity of the HSC compartment to enhanced cycling in steady state. Although HSCs have enormous self-renewal potential after transplantation [57[■]], enhanced HSC cycling in steady state is associated with rapid exhaustion in multiple mouse knockout models in which quiescence of HSCs was disrupted [34[■]]. The role of quiescence in HSC maintenance is not well understood, however, as in some knockouts, such as *Cdkn2c*^{-/-} mice, enhanced HSC cycling was not associated with exhaustion [58]. It has been proposed that quiescence is required to prevent inappropriate differentiation, and therefore loss of HSCs [34[■]]. Thus, maintaining low levels of self-renewal in steady state and favoring attempts at repair over disposal of damaged HSCs could lead to an HSC compartment that expands with age and maintains its overall function, but in which the function of individual HSCs is compromised.

THE ROLE OF DEVELOPMENTAL PROGRAMMING

The progressive myeloid bias of the HSC compartment, which may be caused by the higher intrinsic self-renewal capacity of myeloid-biased HSCs [11,12], likely serves an evolutionary purpose as well. Benz *et al.* [57[¶]] showed that although in fetal liver balanced (β) HSCs predominate, the frequency of myeloid-biased (α) HSCs increases as development proceeds. CLPs derived from α -HSCs were defective and expressed lower levels of B-lineage-specific genes, a phenotype similar to that of aged CLPs [57[¶]]. The adaptive immune system develops early in life to establish antigen-specific responses to the multitude of antigens encountered

during extrauterine life, while preventing responses against self. The cost of this process is iterative recombination of T and B-cell receptor loci accompanied by massive proliferation and deletion of the majority of the cells, which carries the risk of malignancy. Indeed, acute lymphoblastic leukemia is predominantly a disease of children [21]. Once the immune repertoire is established, cessation of recombination and establishment of vigorous and rapid memory responses likely benefits fitness. Thymic involution should probably be viewed in this context as well. Supporting this notion is the fact that not only changes in the types of B and T cells produced [59], but also the decrease in B-cell development [59], the emergence of α -HSC clones [57[¶]]

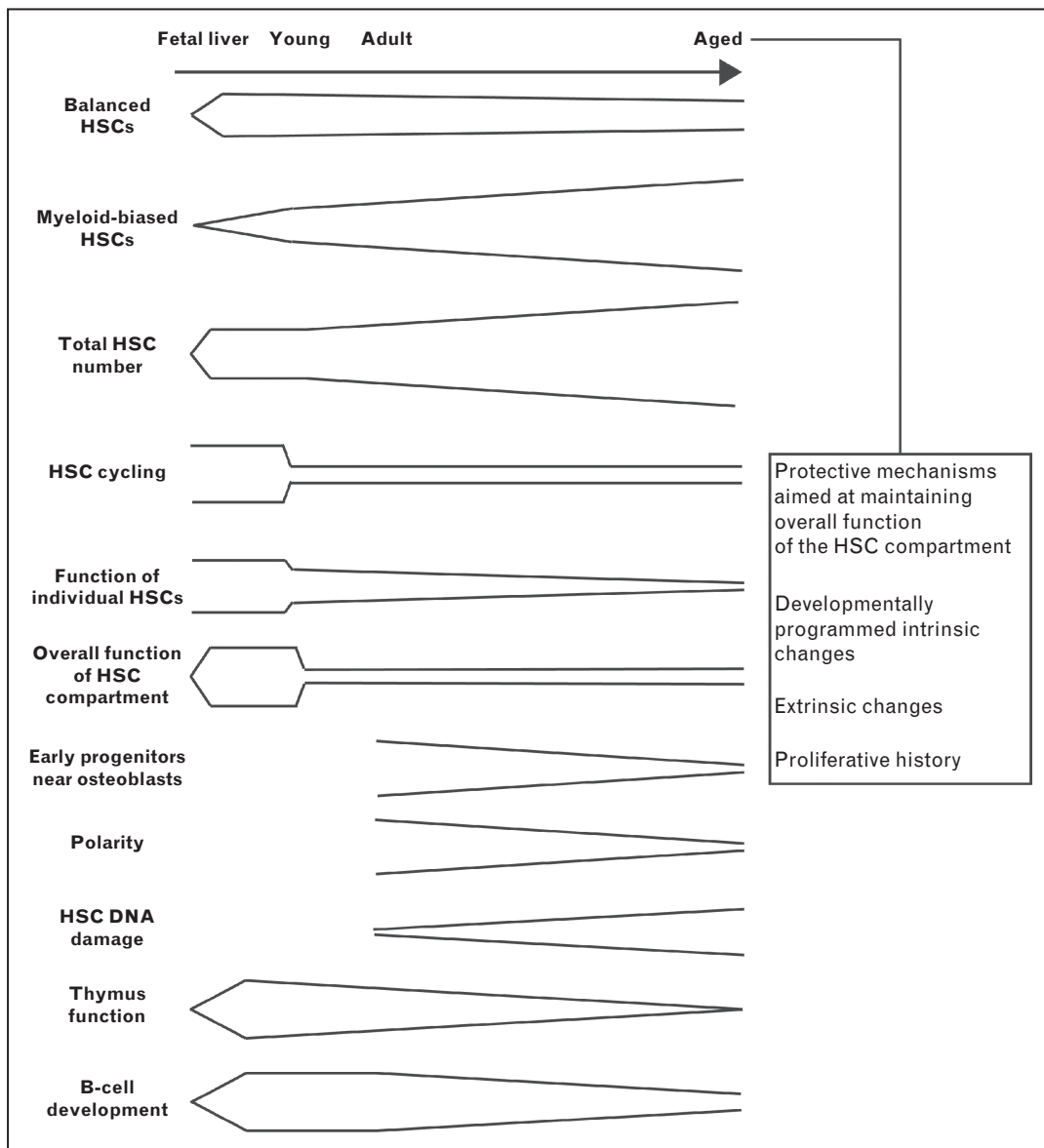


FIGURE 1. Schematic overview of functional changes in the hematopoietic system throughout development and aging. HSC, hematopoietic stem cell.

and thymic involution [59–61] start very early in life, and are therefore likely a result of developmental programs, and not of aging *per se*.

It has recently been shown that most HSCs reside in the perivascular bone marrow niche, whereas very early lymphoid progenitors reside in the osteoblastic niche [62[■],63[■]]. It is not known whether α -HSCs and β -HSCs occupy distinct niches, but it is possible that the localization of aged early progenitors farther away from the osteoblasts is a reflection of a shift toward myelopoiesis [15]. Furthermore, although the argument has been made that epigenetic changes contribute to aging of stem cells [14[■],27,64], epigenetic regulation is critical for development. The generation of induced pluripotent state cells (iPSCs) from somatic cells involves near-complete epigenetic rewiring of the cells toward a developmental ‘ground’ state [65]. The recent demonstration that HSCs in mice derived from iPSCs generated from aged HSCs are functionally ‘young’ suggests that aging of HSCs carries a large epigenetic component and is reversible. The argument could be made, as was acknowledged by the authors, that the data may be a reflection of the selection of a subset of HSCs that are not or are less affected by age-associated damage [66]. However, Florian *et al.* [30[■]] showed that pharmacological inhibition of the RhoGTPase Cdc42 allowed rejuvenation of HSC function in terms of polarity, long-term repopulation capacity and localization closer to the osteoblastic niche. The fact that the Ach4K16 staining pattern also appeared ‘younger’ suggests a reversal of epigenetic changes, which could at least in part be developmentally programmed.

CONCLUSION

Although it is possible that proliferative history contributes to the aging of HSCs, part of the functional phenotype of the aged hematopoietic system may be the result of intrinsic and extrinsic developmental and stem cell-protective mechanisms aimed at maximizing fitness during reproductive life (summarized in Fig. 1). Hematopoietic aging might therefore be an example of antagonistic pleiotropy, which posits that traits that confer fitness prior to and during the reproductive age can be detrimental after reproductive age and contribute to aging [67]. Hematopoietic regulation is geared toward maintaining a functional HSC compartment with optimal homeostatic responses to stress and toward generating an adaptive immune system early in life while avoiding lymphoid malignancies during reproductive age. These processes may decrease fitness later in life, however, as subfunctional; damaged HSCs accumulate and

establishment of responses to neoantigens (tumor-associated or infectious) becomes problematic because of the reduced generation of naive T and B cells.

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Conflicts of interest

There are no conflicts of interest.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 397–398).

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