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Aging of hematopoietic stem cells: Intrinsic changes or micro-environmental effects?

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During development hematopoietic stem cells (HSCs) expand in number and persist throughout life by undergoing self-renewing divisions. Nevertheless, the hematopoietic system does not escape the negative effects of aging, suggesting that self-renewal is not complete. A fundamental issue in stem cell biology relates to such age-dependent loss of stem cell activity. Both stem cell intrinsic factors and extrinsic factors associated with an aging micro-environment could contribute to aging of the hematopoietic system. Recently, changes in the clonal composition of the HSC compartment during aging have been put forward as a key factor. Here, we discuss these recent developments and speculate how they may be of clinical relevance.

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Introduction

Throughout the lifespan of an organism, hematopoietic stem cells (HSCs) within the bone marrow are capable of replenishing all cell types of the blood. This feature renders the bone marrow one of the most highly self-renewing tissues of the body. Nevertheless, the hematopoietic system does not escape the detrimental effects of the aging process. These aging effects are clinically manifested by an increase in the incidence of myeloproliferative diseases, including leukemia [1,2], a decline in adaptive immunity [3–5] and a greater propensity to anemia [6,7]. Moreover, older patients with acute myeloid leukemia (AML) show a lower frequency of favorable core-binding chromosomal abnormalities, a higher incidence of complex aberrant karyotypes and a different gene expression pattern compared to young AML patients [8,9], suggesting differences in underlying biology in old versus young patients. Alterations in the hematopoietic system in response to aging have recently been discussed in some excellent reviews [10•,11,12].

In general, aging is accompanied by a diminished capacity to adequately maintain tissue homeostasis and to repair tissues after injury, suggesting an imbalance in cell loss and renewal. In the hematopoietic system, a reduction in the repopulating capacity of old murine HSCs versus their younger counterparts is observed [13–15]. However, it was also observed that aged bone marrow is still able to repopulate the blood system after serial transplantations [16,17] and HSCs seem therefore able to largely overcome the negative effects of normal aging. Moreover, bone marrow failure is a rare condition in both rodents and humans and even among the most elderly rarely observed.

Since functional hematopoiesis is completely dependent on a small population of HSCs, age-related changes of the hematopoietic system must be the result of age-related alterations in the function of HSCs. As HSCs do not function in isolation, but rather exert their activity in the context of supporting stromal elements in the bone marrow, it is highly likely that both intrinsic and micro-environmental factors contribute to aging of HSCs. This review is aimed to highlight recent developments in the understanding of both intrinsic changes and on the increasing evidence of micro-environmental effects in the process of HSC aging. Surprisingly, data on the effects of aging on human HSCs are rare. Nevertheless, we relate findings from murine model systems to human biology, and speculate on their clinical consequences.

Aging of the hematopoietic system

An ever-increasing number of studies using murine models have investigated the effects of age on the hematopoietic system. Collectively, these studies have made it clear that the hematopoietic system undergoes substantial changes with increasing age. One of the most striking features is a skewing toward a more myeloid-biased output. In mice, changes in lineage potential during aging show a relative decrease in lymphoid output, whereas the myeloid potential is maintained or even increased [14,15,18]. These data are in line with an increasing incidence of myeloid leukemias and a diminished adaptive immunity in aged humans. However, studies investigating potential age-associated lineage

skewing in the human hematopoietic system are still lacking. Another well-documented feature of the aged hematopoietic system in mice is the relative increase in phenotypically defined HSCs [13–15,19,20]. This has recently been confirmed in humans, defining human HSCs by a $CD34^+CD38^-$ [21] or a more stringent $lineage^-CD34^+CD38^-CD90^+$ [10•] phenotype. In mice, however, this increase in HSCs is accompanied by a loss of functional activity. Although considerable variation between mouse strains has become evident, a decrease in competitive repopulating ability was observed in old versus young murine HSCs [13–15]. Whether such functional decline in HSC activity is also present in the human system has still to be elucidated. In a large cohort of matched unrelated allogeneic stem cell transplantations young donor age was associated with better overall survival of the recipient, but no direct effect of donor age on neutrophil engraftment was seen [22]. On the other hand, the propensity to anemia that is often observed in elderly suggests a decrease in functional activity. Detailed studies of individual human HSCs are needed to draw firm conclusions. Unfortunately, these studies in humans are still limited by suboptimal stem cell assays using limiting dilution experiments, limited data on stringent purification of human HSCs, and the mere availability of old human HSCs for research purposes. In many studies human cord blood samples are used, as these are often readily available. We would argue that this is a far from optimal cell source for aging studies. Further, one should realize that the golden standard for the validation of human stem cell properties, the xenotransplantation model, also has its limitations due to species-related differences in biology.

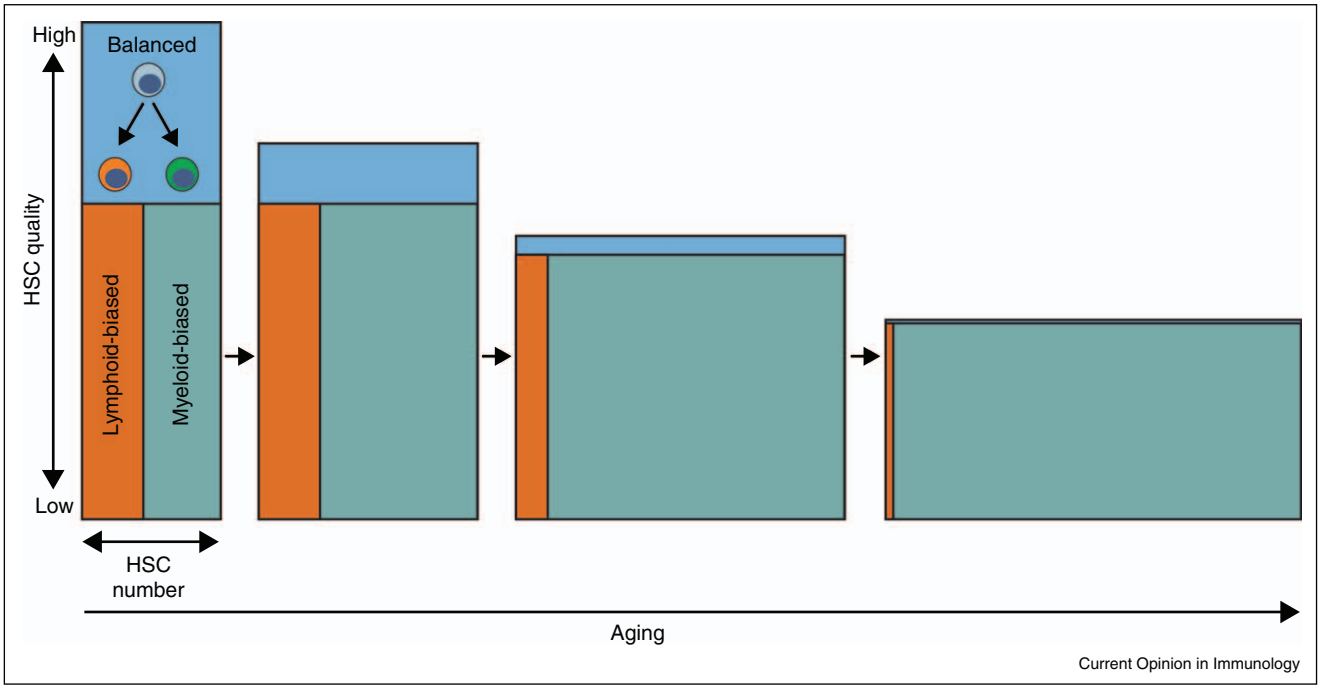
Intrinsic changes of hematopoietic stem cells during aging

As HSCs reside in the bone marrow in close proximity to non-hematopoietic cellular elements, it is highly likely that age-associated alterations in HSCs are due to a combination of both intrinsic and environmental effects. However, the majority of data on HSC aging concern intrinsic changes, often due to the fact that young mice were used as recipients. A prevailing hypothesis that aging of an organism is the result of increasing efforts of cells and tissues to cope with accumulating global damage also seems to hold true for HSCs. During aging, tumor suppressor pathways are activated in response to unavoidable exposure to damaging agents, like reactive oxygen species. Indirect evidence for the involvement of DNA repair mechanisms in aging comes from murine studies. Mice deficient in several genomic maintenance pathways including nucleotide excision repair, telomere maintenance, and non-homologous end-joining show alterations in number and functional decline of HSCs. Some of the phenotypes are reminiscent of normal aging [23]. Also, an increase of γ-H2AX DNA foci (indicating DNA damage) in aged wild type HSCs was demonstrated [23]. This age-associated accumulation of DNA damage was recently also observed in human hematopoietic stem and progenitor cells [24•]. In addition, it was shown in other stem cells that tumor suppressor pathways are activated, including those mediated by p53 and p16 [25,26]. In HSCs, the classical cyclin-dependent kinase inhibitor $p16^{INK4a}$ increases with age and modulates specific age-associated HSC functions [27]. At older age $p16^{INK4a-/-}$ mice had significantly more HSCs, had more dividing cells, and were better able to reconstitute an immune system than wild type HSCs from mice of the same age [27]. The $p16^{INK4a}$ pathway also seems to play a role in human hematopoietic aging, since an increased expression of $p16^{INK4A}$ during aging in human healthy $CD34^+$ hematopoietic cells was demonstrated [8]. Interestingly, an inverse pattern of $p16^{INK4A}$ expression was shown in patients with AML [8,28], suggesting that suppression of the (age-associated) $p16^{INK4A}$ pathway may facilitate leukemogenesis.

As discussed above, skewing in the lineage potential of the HSC population towards a more myeloid-biased potential is one of the most prominent features of the aged hematopoietic system. Recently, it has become clear that heterogeneous HSC populations with different lineage potential (lymphoid-biased, myeloid-biased, or balanced) co-exist in the bone marrow and co-ordinately give rise to hematopoiesis [29••,30••,31,32]. Studies using extensive single cell transplantations of highly purified stem cells demonstrated that the clonal contribution to the different blood cell lineages varies significantly in young mice, and can be stably maintained throughout serial passaging, providing evidence that the pool of HSCs comprises distinct clonal subtypes with differential lineage and self-renewal potential [32,33]. The lineage biased HSCs can be purified based on differential expression of CD150 (Slamf1) [29••]. Within the long-term repopulating $lineage^-Sca1^+c\text{-}kit^+Flt3^-CD34^-$ HSC compartment cells with distinct expression of CD150 can be identified: myeloid-biased HSC are $CD150^{high}$, whereas HSCs with a balanced lineage output are $CD150^{low}$. During aging the $CD150^{high}$ HSC population expands while the $CD150^{low}$ HSC population diminishes, suggesting that they are differentially regulated [29••]. Although these data suggest clonal selection as the predominant mechanism contributing to aging of HSCs, they do not exclude the possibility that deficiencies within defined clonal subtypes also contribute to age-dependent changes. Indeed, the observation that the total repopulating potential diminishes with age in both $CD150^{high}$ and $CD150^{low}$ HSCs suggests that besides clonal selection, aging of HSCs also occurs [29••] (Figure 1). The proposed concept of clones with distinct functional potential has not yet been supported (nor refuted) by experimental evidence in humans.

An alternative, albeit not mutually exclusive, mechanism contributing to age-associated changes of the hemato-

Figure 1



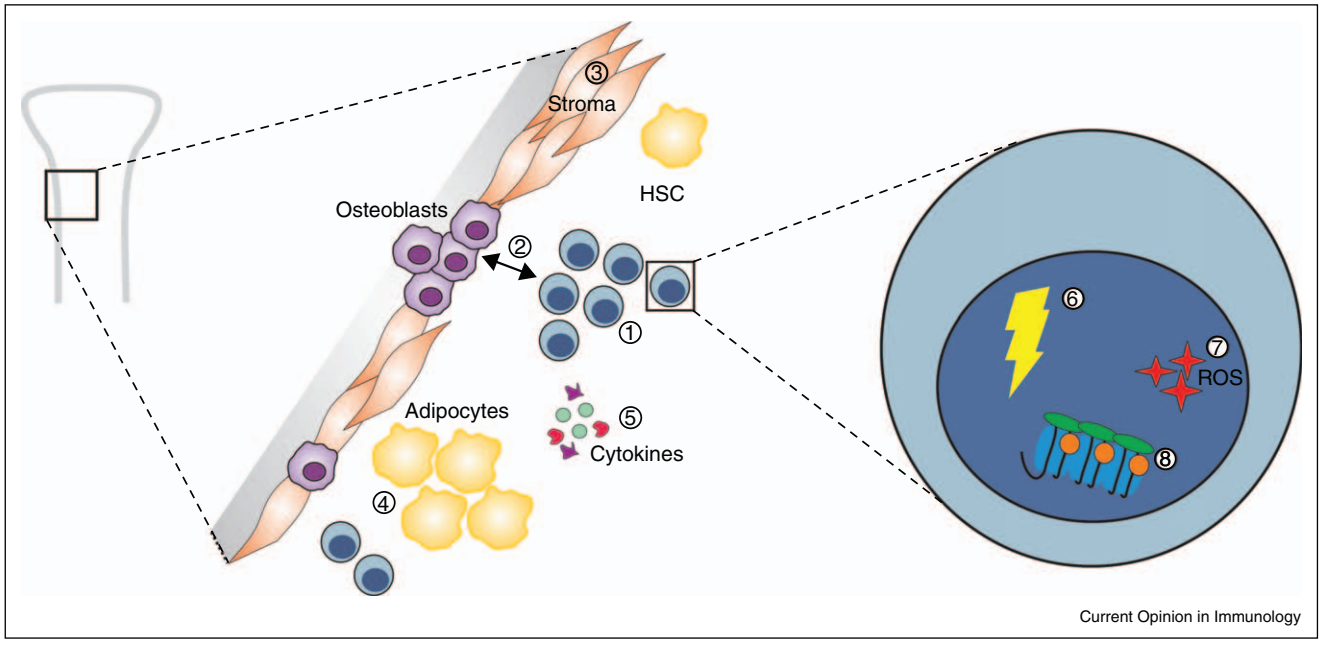
With increasing age the clonal composition within the HSC compartment changes. A proportional shift in lineage potential from balanced to myeloid-biased is observed. Moreover, while there is an increase in HSC number, the per-cell quality of HSCs decreases with age.

poietic system is the occurrence of gradual changes within all HSCs. From studies investigating B-cell aging it has become clear that aging affects the hematopoietic system with considerable inter-individual variation, even in genetically identical and co-housed mice [34–36]. This often-overlooked fact strengthens the importance of non-genetic factors in aging, for example, epigenetic changes. Indeed, monozygotic twins show remarkable differences in epigenome at older age [37]. Gene expression profiling of highly purified long-term HSCs from young and old mice revealed consistent downregulation of genes mediating lymphoid specification and function. In contrast, genes mediating myeloid specification and function were upregulated, strongly suggesting that the changes in lineage potential are underwritten by age-dependent changes in gene expression at the stem cell level [14]. Why and how these gene expression changes are initiated remains fully unclear. Another study revealed that old murine HSCs show high-order changes in gene expression. Groups of genes, or even entire chromosomal regions, which were normally silenced in young HSCs, were activated in old HSCs, whereas conversely other loci were expressed at lower levels than in young cells [13]. Among the genes with reduced expression were those involved in modulating chromatin, suggesting that epigenetic alterations may accumulate in old cells. It is important to realize that these studies compared large populations of HSCs derived from old and young mice, and did not take into account potential population dynamics within or between individual mice. Gene expression profiling studies on human young and old purified HSCs are still lacking, most likely because of the difficulties associated with obtaining cells from healthy old donors.

Micro-environmental effects of the bone marrow during aging

Besides stem cell intrinsic factors also micro-environmental (extrinsic) factors might determine the functional capacity of stem cells in the aging organism. Contribution of micro-environmental effects is especially reasonable in the hematopoietic system, in which the dependence of HSCs on the bone marrow stromal environment (the HSC niche) is very well documented [38–40]. An early study using subcutaneous implantation of bones from young or old mice demonstrated decreased repopulation of young cells into the old bone grafted onto young mice [41]. Similarly, *in vitro* long-term bone marrow cultures on stromal cells derived from either young or old mice have demonstrated a reduced ability of the old stroma to support hematopoietic progenitor cells [42]. Using time-lapse 2-photon microscopy and complex image analysis algorithms it was shown that aged HSCs and early progenitors display a higher cell protrusion activity and are localized more distantly from the endosteum compared to their young counterparts [43•]. This corre-

Figure 2



Overview of proposed age-associated micro-environmental (extrinsic) and intrinsic changes of the hematopoietic system: first, increase in the number of phenotypically defined HSCs; second, more distant localization of HSCs from the endosteum; third, less supportive stroma; fourth, accumulation of adipocytes in the bone marrow; fifth, different cytokine milieu; sixth, increased DNA-damage; seventh, increased exposure to reactive oxygen species (ROS); and eighth, changes in gene expression and accumulation of epigenetic alterations.

lated with reduced adhesion to stroma cells as well as reduced cell polarity upon adhesion of aged HSCs, suggesting altered niche biology in aging. The reduced adherence of HSCs with stroma cells is also suggested by the observation in a murine model that approximately fivefold more HSCs were mobilized after treatment with granulocyte growth stimulating factor (G-CSF) [44]. Conversely, it has been well documented that old HSCs display homing deficiencies upon transplantation in old recipients [45]. Whether these properties of old HSCs are also present in human has never been studied in detail.

The mechanisms underlying the proportional shift in lineage potential seen with age are not understood. One possibility would be a differential response to the aging cytokine milieu. This hypothesis is supported by the demonstration that lineage-biased HSC subtypes respond differently to transforming growth factor β1 (TGF-β1) [30••]. It was shown *in vitro* as well as *in vivo* that TGF-β1 stimulates myeloid-biased HSCs to proliferate while exerting inhibitory effects on lymphoid-biased HSCs, illustrating the unique responsiveness of distinct HSC subtypes to a growth factor and providing a potential mechanism for differential regulation of HSC subtypes [30••]. It could be speculated that the inflammatory setting of an aging environment could be the setting which causes the increase of the myeloid-biased HSCs. In line with this hypothesis, might also be the observation of reduced cellularity in the bone marrow of human elderly [46]. Together with the observations in murine models that bone marrow adipocytes accumulate with age [47] and that these adipocytes are negative regulators of the bone marrow micro-environment [48], it could be hypothesized that adverse effects of the aged bone marrow composition impact HSCs.

Analogous to experiments which demonstrated that the age-related decline in hepatocyte progenitor cell activity can be modulated by systemic factors that change with age [49] the heterochronic parabiosis mouse model was used to study the effect of systemic signals on hematopoiesis [50]. However, this last paper was retracted after publication and conclusions should therefore be considered with caution. It remains to be determined to what extent the adverse effects of age on HSC functioning are reversible. Detailed studies on the molecular causes of HSC aging will be required to assess the feasibility of reversibility.

Conclusion

Aging of the hematopoietic system is accompanied by declining immunocompetence, increased incidence of anemia and increased predisposition to myeloid leukemias. Two models have been put forward to account for the changing functional properties of the aging HSC pool. In one model the functional potential of stem cell clones

within the pool changes over time because of gradual alterations that occur in all HSCs. Alternatively, the clonal composition of the functional stem cell pool is different in older individuals compared to younger individuals, while individual HSCs do not age. However, both models are not mutually exclusive. Clonal studies at the single cell level will be required to distinguish between these scenarios. Moreover, it is likely that these changes are at least partly influenced by micro-environmental effects (Figure 2), of which we understand very little. Although it is evident that the prevalence of hematological diseases increases with age, it is unclear whether the observations made in aged murine HSCs are also evident in humans and contribute to the initiation of disease.

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