Review

Stem cells and aging in the hematopoietic system

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The effector cells of the blood have limited lifetimes and must be replenished continuously throughout life from a small reserve of hematopoietic stem cells (HSCs) in the bone marrow. Although serial bone marrow transplantation experiments in mice suggest that the replicative potential of HSCs is finite, there is little evidence that replicative senescence causes depletion of the stem cell pool during the normal lifespan of either mouse or man. Studies conducted in murine genetic models defective in DNA repair, intracellular ROS management, and telomere maintenance indicate that all these pathways are critical to the longevity and stress response of the stem cell pool. With age, HSCs show an increased propensity to differentiate towards myeloid rather than lymphoid lineages, which may contribute to the decline in lymphopoiesis that attends aging. Challenges for the future include assessing the significance of ‘lineage skewing’ to immune dysfunction, and investigating the role of epigenetic dysregulation in HSC aging.

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1. Introduction

Although the blood is the definitive self-renewing tissue of the body, it does not escape the detrimental effects of the aging process. Hematopoietic aging is manifested in human populations in the form of an increase in myeloproliferative disease, including leukemias (Lichtman and Rowe, 2004), declining adaptive immunity (Gruver et al., 2007; Hakim and Gress, 2007; Linton and Dorshkind, 2004), and greater propensity to anemia (Beghe et al., 2004; Guralnik et al., 2004). Since we rely on a small reserve of hematopoietic stem cells (HSCs) to replenish all the cell types of our blood throughout life, it seems reasonable that these defects could trace back to the aging of the HSC pool. At the same time, bone marrow failure is exceedingly rare even among the most elderly, which implies that stem cell exhaustion does not accompany normal aging. Studies conducted in mice over 20 years ago showed that aged bone marrow donors can repopulate the blood in serial transplants spanning multiple lifetimes, prompting the hypothesis that the HSC is effectively ageless (Harrison, 1979, 1983). Recent work provides us with a complex and still-incomplete picture of the interplay between the aging of the HSC pool and that of the blood proper (Fig. 1). As is true of other tissues, the incidence of cancer in the blood increases steeply with age (Edwards et al., 2002). There is reason to believe that stem cells have an important role in this process, as incubators for the multiple hits required for oncogenesis, and perhaps also because their capacity for replication without differentiation poises them close to a cancer phenotype (reviewed in Rossi et al., 2008). While genomic damage is inextricably linked to cancer, it remains unclear to what extent age-dependent DNA damage accrual in the stem cell pool contributes to other aspects of aging in the hematopoietic system. Mutant mice with defects in diverse DNA repair pathways or telomere maintenance do evidence diminished stem/progenitor cell function, particularly under conditions of stress and regeneration, demonstrating that life-long stem cell maintenance depends on these functions (Carreau et al., 1999; Haneline et al., 1999; Navarro et al., 2006; Nijnik et al., 2007; Noll et al., 2002; Prasher et al., 2005; Reese et al., 2003; Rossi et al., 2007a; Samper et al., 2002). The role played by epigenetic changes in stem cell aging, whether developmentally regulated or stochastic in nature, is only beginning to be addressed. Microarray analysis has uncovered a shift in the gene expression profile of the HSC with age, with genes associated with myeloid differentiation programs upregulated, and genes specifying lymphoid fate and function downregulated (Rossi et al., 2005). Such transcriptional changes may underlie the characteristic lymphoid/myeloid lineage skewing of aged HSCs and contribute to the decline in adaptive immunity in old age (Kim et al., 2003; Liang et al., 2005; Rossi et al., 2005; Sudo et al., 2006). Alterations in the propensity of HSCs to produce myeloid cells and erythrocytes may also be involved in the increase in myeloproliferative disorders and anemia seen in the elderly, although here the case is still weak.

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2. HSC function and phenotype

The HSC was the first stem cell to be identified, and remains the best-studied tissue-specific stem cell. Isolation and characterization of the HSC has been facilitated by the development of adoptive transfer techniques, which afford a gold standard test for long-term self-renewal and multilineage potential. In these transplantation assays, bone marrow isolates are intravenously injected into conditioned recipient animals to evaluate their ability to reconstitute the blood. Such experiments have shown that, while the bone marrow contains abundant progenitor cells which can transiently regenerate some or all blood lineages, only a tiny fraction of these cells has long-term multilineage reconstituting (LTMR) activity, the defining characteristic of the HSC (Spangrude et al., 1988). Even a single cell of this true stem cell fraction can rescue a radioablated host (Osawa et al., 1996). Differences in developmental potential and lineal relationships between stem and progenitor subsets establish a hierarchical structuring of the primitive hematopoietic compartment, with HSCs giving rise to multipotent progenitors, which in turn give rise to progenitors with more restricted lineage potentials that ultimately differentiate into all the effector cell types of the blood (reviewed in Bryder et al., 2006). In order to sustain the stem cell pool and hence their ability to replenish the blood, HSCs have the ability to self-renew through mitoses that yield at least one daughter stem cell. It is thought that capacity for long-term self-renewal is unique to the HSC within the hematopoietic compartment, whereas downstream progenitor populations have only a limited ability to proliferate without undergoing further differentiation.

In the mouse, long-term repopulating activity is highly enriched in the fraction of bone marrow cells expressing the receptors c-kit and Sca-1, and lacking expression of membrane proteins specific to more differentiated blood lineages. This c-kit+ lineage− Sca-1+ (KLS) compartment encompasses virtually all the LTMR activity of the bone marrow (Bryder et al., 2006). However, true stem cells comprise only a small fraction of the KLS population; most of the cells isolated by the KLS criteria are early downstream progenitors with transient or negligible capacity for self-renewal (Bryder et al., 2006). In the hematopoietic literature, the KLS compartment is sometimes divided into long-term reconstituting HSCs (LT-HSCs), short-term reconstituting HSCs (ST-HSCs), and multipotent progenitors (MPPs). Since the ST-HSC is more accurately classed as a type of multipotential progenitor rather than a stem cell, we here use the term HSC to refer exclusively to the long-term reconstituting fraction.

A number of cell-surface markers have been identified which enable higher enrichment of HSCs than can be achieved using the KLS criteria alone. In the mouse, Thy-1lo, CD34−, Flk2− (Flt3−), endoglin+ (CD105+), and Slam+ (CD150+) KLS sub-fractions all give improved HSC purity (Adolfsson et al., 2001; Chen et al., 2002; Christensen and Weissman, 2001; Kiel et al., 2005; Osawa et al., 1996; Spangrude et al., 1988). HSC can also be enriched by virtue of their ability to efflux intravital staining dyes such as rhodamine-123 (Bertoncello et al., 1985) and Hoechst 33342 (Wolf et al., 1993). Single HSCs isolated using the most selective marker panels can long-term reconstitute irradiated hosts with 40% or higher efficiency (Ema et al., 2000; Kiel et al., 2005; Takano et al., 2004; Yamazaki et al., 2006). Integration of the data from the HSC literature is still hindered by the fact that, especially in early works, the population designated HSC must presumably include a superabundance of MPPs, which have enormous developmental potential and yet are not true stem cells. This is particularly problematic in the context of aging studies, as the frequency and absolute numbers of HSCs and downstream progenitors in the bone marrow changes dramatically with age (Morrison et al., 1996; Rossi et al., 2005; Sudo et al., 2000).
3. The HSC pool from ontogeny to old age

The character of the stem cell pool undergoes profound changes over the course of ontogeny (reviewed in Kondo et al., 2003; Mikkola and Orkin, 2006). Fully multipotent HSCs originally emerge in the aorta–gonad–mesonephros (AGM) region of the early embryo, possibly as the result of maturation of more restricted progenitors seeded there from the yolk sac (Samokhvalov et al., 2007). The liver is the primary site for HSC production and residence within the developing fetus. Fetal liver HSCs undergo rapid doubling divisions during late embryogenesis (Morrison et al., 1995a), and begin to colonize the bone marrow shortly before birth (Christensen et al., 2004). This migration is accompanied by changes in HSC surface phenotype (Morrison et al., 1995a), differentiation potential (Douagi et al., 2002), and transcriptional regulation (Kim et al., 2007). The stem cell pool expands rapidly after birth and then quiesces into something approximating a steady state (Bowie et al., 2006). In mature C57BL/6 (B6) mice, it has been estimated that 0.004% of mononuclear cells in the bone marrow are HSCs (Otsawa et al., 1996). HSC pool size varies by as much as sevenfold across common laboratory mouse strains (de Haan and Van Zant, 1997; Morrison et al., 2002). The frequency of bone marrow cells with the HSC surface phenotype increases several-fold with age in B6 mice (Morrison et al., 1996; Nakauchi et al., 1999; Pearce et al., 2007; Rossi et al., 2005; Yilmaz et al., 2006). In adoptive transfer trials, the LTMR activity of the stem cell population shows a more modest, twofold increase in aged B6 mice (Harrison, 1983; Harrison et al., 1989). By the same measure, the functional HSC pool declines in old age in a number of short-lived mouse strains, including CBA, DBA and BALB mice (Chen et al., 2000; Harrison, 1983).

In principle, HSCs can undergo three distinct types of mitosis: (1) symmetric divisions producing two daughter stem cells, (2) asymmetric divisions producing a replacement stem cell and a differentiating progenitor, and (3) symmetric divisions yielding two differentiating progenitors. Since stem cell numbers increase – rapidly during fetal development, and slowly in maturity – self-renewing symmetric divisions must occur with some frequency throughout life. Both symmetric and asymmetric division of HSCs have been demonstrated using sophisticated pair-daughter experiments and transplantation assays (Ema et al., 2000; Takano et al., 2004). The observation that HSC numbers change only slowly after maturity is consistent with the bulk of their mitoses being asymmetric, but balanced differentiating and non-differentiating symmetric divisions could give the same result. Pool dynamics could also be affected by programmed cell death within the HSC population. It is not clear to what extent apoptosis occurs in the stem cell pool, but mice which over-express the anti-apoptotic gene Bcl2 show a twofold expansion of the KLS Thy1$^{lo}$ compartment, and these cells exhibit improved plating efficiency and out-compete wild-type cells in co-transplantation assays (Domen et al., 2000).

The kinetics of HSC cycling in the steady state is still a matter of debate. Early work tended to favor a stratified, ‘clonal succession’ model in which most of the HSC pool remains quiescent in G0 while a small fraction of stem cells divide to replenish the blood (reviewed in Morrison et al., 1995b). More recently, BrdU labeling studies have challenged this idea, suggesting rather that the HSC pool is slowly cycling with fairly uniform kinetics (Cheshier et al., 1999; Kiel et al., 2007; Passegue et al., 2005). One recent study which used stringent phenotypic criteria for HSC isolation (KLS CD150$^{+}$ CD48$^{-}$ CD41$^{-}$) estimated that the rate of entry into G1/S/G2/M cycling is 6% per day (Kiel et al., 2007). Still, studies based on Ki-67/Pyrinon-Y staining and DNA content have put the fraction of stringently defined HSCs in G0 at over 90% in adult mice, while showing a sharp increase in cycling in immediate downstream progenitors (Rossi et al., 2007b; Yamazaki et al., 2007). The regulation of stem cell quiescence, differentiation, and self-renewal is subject to significant genetic variation across mouse strains. In allotrophic B6→DBA mice produced from embryo fusion chimeras, B6-derived cells predominate in the peripheral blood after maturation, but DBA-derived hematopoiesis is transiently boosted in a transplant setting and following administration of the hematopoietic cytokine SCF (Phillips et al., 1992; Van Zant et al., 1992). Since the LTMR activity of the HSC pool is more robust with age in the B6 strain (Chen et al., 2000), these findings hint that a trade-off may exist between rapid HSC proliferation to meet hematopoietic stresses and long-term maintenance of stem cell function.

Because undifferentiated HSCs cannot yet be sustained or robustly expanded in vitro, the effects of extrinsic factors on their division and fate choice are poorly understood, and the determinants of HSC pool size remain unclear. It has been argued that the size of the stem cell pool is probably not under feedback regulation, based on the inter-strain variability in HSC frequency, the tendency of the pool to expand slowly with age, and the observation that reductions in pool size following irradiation or chemotherapy can persist long-term (de Haan and Van Zant, 1997). The results of a quantitative serial transplantation study conducted by Iscove and Nawa support homeostatic regulation of HSC pool size (Iscove and Nawa, 1997). In these experiments, the stem cell pool established on primary transplant to a radioablated host stabilized at around 5–10% of the normal level after 6 months, measured in terms of LTMR activity. The plateau level displayed only a weak dependence on the number of bone marrow cells transplanted—instead, HSC expansion within the host correlated inversely with HSC dosage. While the plateau LTMR activity fell further in secondary and tertiary transplant recipients, these declines were not nearly as dramatic as on the first transplant. Together, these observations suggest that (1) HSC pool size is feedback regulated and (2) the difference between the steady state pool size in normal and transplanted animals is mainly a function of reconstitution kinetics in the transplant setting. The authors of the study proposed a model in which cytokine levels read out bone marrow cellularity to negatively regulate HSC self-renewal. According to this model, the host bone marrow fills up rapidly owing to the presence of highly proliferative progenitors in the transplant, and the HSC pool consequently switches from expansion to steady state at a plateau level lower than is attained in normal ontogeny.

In the steady state, a small fraction of the total HSC pool is dispersed within the peripheral blood and the spleen. The dynamics, regulation and function of stem cell trafficking between the bone marrow niche and the blood are only beginning to be characterized (reviewed in Kaplan et al., 2007), and little is known about the effects of aging on this process.

Most blood effector cells have very short post-mitotic lifetimes compared to the lifespan of the animal, with the exception of memory B and T cells, which in a sense constitute antigen-specific stem cells of adaptive immunity. The murine stem cell pool can only generate a few thousand immediate downstream progenitors per day (Domen et al., 2000), so these progeny must undergo massive clonal expansion to replace the millions of effector cells lost in the periphery every day. There is evidence that extensive apoptotic attrition occurs during the early steps of differentiation/proliferation (Necas et al., 1998). This attrition may be part of the homeostatic mechanism which matches progenitor supply to lineage-specific demands, but could also have a role in purging genetically damaged and potentially oncogenic precursors from the blood.
4. Effects of age on HSC regenerative potential

Early experiments conducted by Harrison et al. showed that bone marrow cells transplanted from aged B6 donors into young, congenitally anemic W/\textsuperscript{w} mice could rescue the erythropoietic defect long-term. Further, this result could be repeated in serial transplantation experiments spanning several lifetimes (Harrison, 1979). The same researchers found that, while bone marrow stem cell activity was eventually exhausted by multiple rounds of serial transplantation, the number of passages required to reach this limit was barely affected when the stem cells were aged in vivo between transfers (Harrison et al., 1978). Even if the stem cell pool retains robust repopulating activity in old age, it does not necessarily follow that the HSC itself is ageless. Several studies which have looked at reconstituting activity on a per-HSC basis have measured a decline in potential with age (Morris et al., 1996; Rossi et al., 2005; Sudo et al., 2000). These data indicate that the phenotypic compartment which is highly enriched for HSCs in young animals yields only a fraction of the same activity in adoptive transfer experiments involving aged donors. In B6 mice, the drop in per-HSC potential roughly cancels out the increase in the size of the population with age (Morris et al., 1996). This might suggest that age-dependent expansion of the HSC pool is a compensatory mechanism which props up overall reconstituting activity in old age, trading off HSC quantity for quality. Pool expansion could be a response to demand-side signals or, alternatively, a trend to gradual expansion of the HSC population after maturation could be genetically ‘fixed’ in the program that governs their self-renewal kinetics. Irrespective of the mechanism, the genetic variability of HSC pool size and dynamics across mouse strains shows that such functional compensation cannot be under strong positive selection. This is not altogether surprising, given that B6 mice, at least, seem to retain ample surplus HSC reserve capacity in old age.

In general, many of the studies evaluating per-HSC reconstituting activity with age are subject to the uncertainties already mentioned regarding the quantification and purity of the stem cells assayed. With that important caveat noted, the mechanistic basis for the observed decline in per-cell potential remains unknown. It has been proposed that old stem cells have a deficiency in bone marrow engraftment, on the grounds that their reduced potential is not manifested in ex vivo clonogenic assays (Morris et al., 1996). In support of this hypothesis, when young and old bone marrow cells were seeded into a radioablated host and then retransplanted after only 24 h, the fractional recovery of LIMR activity from the recipient bone marrow was twofold lower for the old HSCs, arguing for a corresponding drop in homing efficiency (Liang et al., 2005). Another study measured a 3.7-fold drop in the per-cell activity of purified HSCs with age when adoptive transfers were done by intravenous injection, but young HSCs had only 1.4-fold advantage over old when the cells were directly injected into bone marrow (Pearce et al., 2007).

5. The role of genomic maintenance

While homing defects may account for some of the age-related decline in per-HSC activity measured in adoptive transfer assays, declining per-cell potential could also signify that aged HSCs are less competent at producing functional progeny because of generalized cellular aging consequent to DNA damage, telomere attrition, and epigenetic dysregulation. These mechanisms could lower per-cell reconstituting potential by promoting apoptosis or permanent cell-cycle arrest (senescence), or by disturbing differentiation and proliferation programs (Fig. 1). Cellular aging could have both chronological and replication-dependent components. It seems possible that the stem cell exhaustion seen during serial transplantation experiments recapitulates and exaggerates the latter. An analysis of the outcomes from primary and secondary HSC transplants showed that prospectively identified stem cells (RB-123<sup>+</sup> Sca-1<sup>+</sup> in Thy-1<sup>−</sup>) established a large pool of phenotypically similar cells in the primary recipients’ bone marrow, indicating at least a thousand-fold proliferation of the donor cells in vivo (Spangrude et al., 1995). Reminiscent of the situation in the aging stem cell pool, the reconstitution obtained when these cells were isolated and transplanted into secondary recipients was very poor compared to that achieved in the first transfer.

That genomic maintenance is critical to sustained HSC self-renewal is implicit in two well-established features of the system: (1) the unusually severe apoptotic response of the HSC pool to irradiation, which enables ablation of the hematopoietic system without killing the host, and (2) the expression of telomerase in the HSC, which is uncharacteristic of somatic cells (reviewed in Harrington, 2004). Certain aspects of HSC physiology could slow the build-up of genomic damage in these cells. Because HSCs are mostly quiescent in G0, their metabolic activity is low, which could reduce their burden of reactive oxygen species (ROS). These metabolic by-products are thought to contribute to DNA damage, especially within the mitochondria (Lindahl, 1993; Sohal and Weindruch, 1996). The bone marrow is a hypoxic environment, which may further reduce the generation of oxygen free radicals (Jang and Sharkis, 2007). Expression of ABC transporters in HSCs is thought to be a mechanism for minimizing DNA damage due to xenobiotic genotoxins (reviewed in Raaijmakers, 2007). It has also been proposed that stem cells can reduce their mutational load by differentially segregating chromosomes during asymmetric divisions, such that the chromosomes which contain older, potentially replication-error free DNA, strands are retained in the stem cell daughter (Cairns, 1975; Conway et al., 2007; Karpowicz et al., 2005; Potten et al., 2002; Shinin et al., 2006). However, this ‘immortal strand’ model has recently been convincingly rebutted, at least in so far as it applies to HSCs (Kiel et al., 2007). Whether cytoprotective mechanisms significantly reduce the burden of mutation in the HSC relative to other cell types remains to be demonstrated. The accumulation rate and spectrum of nuclear somatic mutations have been shown to vary widely across tissues (Vijg et al., 2005), but similar measurements have yet to be made in the HSC, in part because the scarcity of these cells makes such an evaluation technically challenging.

Studies conducted using mutant mice have underscored the importance of DNA repair and telomere maintenance pathways to maintaining the viability of the stem cell pool. DNase Ligase IV (Lig4) is an essential enzyme in the non-homologous end-joining (NHEJ) mechanism which repairs double-strand breaks (DSBs). A recent study investigated hematopoietic defects in mice expressing a hypomorphic Lig4 allele, Lig4<sup>2286C</sup> (Nijnik et al., 2007). Aged Lig4<sup>2286C</sup> mice had reduced bone marrow cellularity compared to controls, and the absolute numbers of KLS cells were diminished in accordance with the reduced size of these mice. Interestingly, the bone marrow frequency of HSC (KLS CD34<sup>+</sup> Flk3<sup>−</sup>) compartment appeared to be relatively undiminished in the Lig4<sup>2286C</sup> mice while more actively cycling multipotent progenitors were diminished. Adoptive transfer experiments showed that the mutant bone marrow had compromised repopulating activity relative to the wild-type, a deficit affecting reconstitution of all bone marrow compartments evaluated.

Similar deficits in reconstituting ability have been identified in mutants with other genomic maintenance defects, as evidenced in a recent study encompassing mice defective in NHEJ (Ku80<sup>−/−</sup>), nucleotide-excision repair (NER) (XPD<sup>−/−</sup>), and telomere main-
tenance (mTR−/−) (Rossi et al., 2007a). Whereas HSC numbers increased with age in the three mutants, as in control animals, all the mutants exhibited a relative decline in the bone marrow frequency of early, cycling downstream progenitors. This reduction in progenitor numbers was not strictly age-related, and was more evident in lymphoid than in myeloid or multipotent progenitors. In competitive transplantation assays, purified HSC from the mutants displayed reduced LTMR activity relative to wild-type, the magnitude of this deficit increasing with donor age. Across the board, mutant HSCs showed reduced self-renewal and increased apoptotic responses in vitro, with differences to controls becoming more pronounced with age. This work also examined the prevalence of γ-H2AX DNA repair foci in wild-type stem and progenitor cells during aging. The number of foci per cell was found to increase sharply with age in HSCs, but fell off in downstream progenitors, with no significant difference between young and old being detectable at the level of myeloid or lymphoid-committed progenitors.

The results of these studies imply that genomic repair ability is a limiting factor in the maintenance of functional HSC reserves. It seems plausible, then, that some of the decline in per-HSC reconstituting potential which attends normal aging could be due to genomic damage or telomere erosion. Developing this idea, we might envisage that, even as the HSC compartment enlarges in old age, the quality of the individual stem cells goes down on average, owing to a silent burden of genomic damage. On this model, loss of potential could be mediated by the activation of apoptotic or cytostatic tumor suppressor pathways at cell-cycle checkpoints, to delete or arrest the growth of defective progenitor clones (reviewed in Rossi et al., 2008). The age-linked expansion of the phenotypically defined HSC compartment relative to the MPP compartment is compatible with cytostatic responses to damage at the HSC level or, alternatively, a delayed or stochastic culling of defective genomes at the level of multipotent progenitors.

While genome maintenance is critical to the longevity of functional stem cell reserves, this does not necessarily entail that genomic instability drives hematopoietic aging at the tissue level. True, as natural selection has little force against defects which manifest only in old age, we might expect mechanisms which preserve cellular function to be good enough to promote survival past reproductive maturity, and not much better than that. But, the same logic also applies to (for example) the signaling networks which regulate cellular, tissue, and systemic homeostasis. It remains an empirical question which failure modes are rate limiting in the decline of any given tissue or species. Regardless of how much genomic damage actually accrues in HSCs, Harrison’s experiments imply that stem cell depletion does not approach a crisis level in normal murine aging (Harrison, 1979). Should we therefore dismiss genomic instability as functionally irrelevant to blood aging phenotypes? Some cautionary data emerges from studies on the role of telomere maintenance in HSCs. Telomere attrition limits the replicative lifespan of many mammalian cell types in culture, and is an obvious candidate explanation for the progressive loss of stem cell potential seen in serial transplantation experiments, given the replicative stress involved in these procedures. In addition to their well-established role as a mitotic clock, there is evidence that telomeres are highly susceptible to ROS-induced DNA damage and function as genomic ‘sentries’ by triggering cell-cycle checkpoint controls in response to oxidative stress (Martin-Ruiz et al., 2004; Passos et al., 2007; von Zglinicki, 2002). Telomere shortening within the HSC has been shown to accompany both normal aging and serial transplantation (Vaziri et al., 1994), and both the rate of telomere loss and the drop in reconstituting potential are accelerated in telomere maintenance deficient (mTR−/−) mice (Allsopp et al., 2003a). Nonetheless, in experiments using a mouse engineered to over-express telomerase reverse transcriptase (TERT), no increase in serial transplantation potential over wild-type was obtained, even though telomere erosion was ameliorated (Allsopp et al., 2003b). Although these findings cannot be safely extrapolated to humans, which have much shorter telomerases than mice (Kipling and Cooke, 1990), they make it doubtful that telomere attrition is central to murine HSC aging in the wild-type setting.

The relevance of DNA damage to normal blood aging is hard to test, as no available treatment or genetic model can reliably lower mutagenic load. Age-related changes within the stem cell compartment and the peripheral blood were apparently rescued by caloric restriction (CR) in BALB mice, a short-lived strain in which HSC function normally declines with aging (Chen et al., 2003). If, as is often argued, the effects of CR are mediated by a reduction in intracellular ROS generation, then this might signal an important role for ROS-induced DNA damage in HSC aging. Indeed, a number of recent studies have provided clear evidence of a role for ROS in the preservation of HSC function. The gene ATM (ataxia telangiectasia-mutated) has recently been shown to be vital for regulating intracellular ROS levels. HSCs from ATM-deficient mice displayed increased ROS levels and a concomitant decrease in functional capacity which could be rescued by administration of the antioxidant N-acetyl-l-cysteine (NAC) (Ito et al., 2004). Loss of HSC activity in ATM-deficient mice is mediated by the activation of p38 MAP kinase and induction of the p16Ink4a tumor suppressor (Ito et al., 2006). In a related study, HSCs from mice lacking Foxo transcription factors (Foxo1/Foxo3/Foxo4 triple mutants) showed increased ROS, reduced LTMR activity, increased cycling, and increased apoptosis (Tothova et al., 2007). Stem cell and progenitor reserves were also reduced in these animals. As in ATM-null mutants, these differences from wild-type could be reversed by antioxidant treatment. Another study showed that Foxo3-deficiency alone was sufficient to diminish HSC function, elevate ROS levels, disrupt stem cell quiescence, and decrease HSC numbers during aging (Miyamoto et al., 2007). Cumulatively, these studies identify management of ROS levels by ATM-p38-p16Ink4a and Foxo transcription factors as a key factor in the preservation of functional HSC reserves over the organismal lifetime.

It is also conceivable that age-linked genomic damage in HSCs produces deleterious changes in the blood via effects more subtle than loss of proliferative capacity. One model for how mutations could promote sub-oncogenic cellular dysfunction invokes the idea that cumulating genomic rearrangements in regulatory DNA disrupt transcriptional regulatory networks (Vig et al., 2005). An across-the-board destabilization of gene regulation was recently found in aging cardiomyocytes, which resembled the effects of genotoxic stress on cultured fibroblasts (Bahar et al., 2006). However, a survey of cell-to-cell transcriptional heterogeneity encompassing HSCs and three terminally differentiated blood cell types found no indication of such an effect in the blood, perhaps reflecting the purging of seriously damaged progenitors during differentiation and proliferation (Warren et al., 2007). Still, the possibility that smaller-scale regulatory disturbances propagate clonally from aged HSCs to the effector cell pool cannot be discounted.

6. Gene expression changes and lineage skewing

Early studies on HSC aging focused on the limits to their replicative potential and the relevance (if any) of genomic instability to this question. During the last few years, there has been increased interest in the idea that changes in the HSC transcriptome contribute to blood aging phenotypes. Microarray analysis of the HSC pushes the limits of the assay as only on the
order of 2000 highly purified stem cells can reasonably be recovered from a single mouse, but new techniques for the global amplification of cDNA have overcome this stumbling block. Comparison of the gene expression profile of young and old HSCs (KLS CD34− Flk2−) showed upregulation of a cadre of myeloid lineage-associated genes and downregulation of lymphoid genes with age (Rossi et al., 2005). The same work evaluated the relative contribution of young and old HSCs to different lineages in competitive transplantation assays. On a per-cell basis, although old HSCs gave poorer long-term reconstitution in all lineages evaluated, B-lymphopoiesis suffered disproportionately. The size of the bone marrow population associated with early, lymphoid lineage progenitors—the common lymphoid progenitor, or Flk2+ CLP (Kondo et al., 1997) was also found to decline progressively with age. This finding is consistent with the results of an earlier study which showed CLP numbers falling steadily after maturity, and demonstrated that cultured CLPs from old animals have reduced proliferative responses to the lymphoid-specific cytokine interleukin 7 (Miller and Allman, 2003). Evidence of a cell-intrinsic, age-related skewing of HSC lineage potential has been found in a number of adoptive transfer studies, although there are still unresolved conflicts in the data. An age-linked shift to myelopoiesis at the expense of lymphopoiesis is most commonly reported, although there is disagreement as to whether the lymphoid deficit is restricted to B-lineage potential (Kim et al., 2003; Rossi et al., 2005), T-lineage potential (Liang et al., 2005), or affects both (Sudo et al., 2000). In contrast to these findings, another study found a profound deficit in short-term B-lineage reconstitution on transplantation of old HSCs (KLS Thy-1+ Mac-1− CD44) but, in limiting dilution assays, the fraction of these cells which yielded long-term multilineage reconstitution gave no evidence of age-related lineage skewing (Morrison et al., 1996).

An HSC-intrinsic drop in lymphoid potential could be secondary to cumulating genomic damage, if downstream lymphoid progenitors are more susceptible to apoptosis or senescence owing to distinctive proliferation kinetics or lower checkpoint thresholds for activation of these pathways. Nonetheless, the observation that lymphoid-specific gene expression declines in HSCs with age suggests that lineage skewing is at least partly a consequence of transcriptional changes (Rossi et al., 2005). Since microarray analyses can only look at population-average mRNA levels, we cannot yet ascertain if these expression changes reflect population-wide shifts, or changes in the internal population structure of the HSC compartment. The report of an expanding stem cell subset which gives rise almost exclusively to myeloid reconstitution on transfer offers some support for the latter model (Sudo et al., 2000). We are also not yet in position to say whether gene expression changes in the HSC compartment are driven by cell-autonomous mechanisms or are induced over time by the microenvironment. Interestingly, there is evidence that diminished B-lineage potential attends serial transplantation as well as aging (Spangrude et al., 1995), which might indicate that lineage skewing has a replication-dependent component. While this could tie lineage skewing to genomic damage rather than gene regulation, it might also be that cell-autonomous changes in gene regulation are ‘clocked’ by cell division, perhaps because of the gradual loss of epigenetic marks (Villeponteau, 1997). A recent microarray analysis of HSC aging found a striking downregulation of genes relating to chromatin remodeling and silencing with age, as well as a doubling of the number of chromosomal loci displaying coordinate changes in gene expression—for the most part, coordinate upregulation (Chambers et al., 2007). It will be interesting to see if changes in HSC expression of lineage-specific genes can be linked to alterations in the epigenome, and if experimentally induced HSC proliferation has any impact in this regard.

7. Discussion

In the quest to understand the aging process, there has been a tendency to seize on newly discovered biological principles and ascribe to them a primary role in this most universal of human afflictions. Glandular atrophy, somatic mutation, and telomere exhaustion have successively taken stage center in speculative unified theories of aging. In the last few decades, insights from evolutionary theory have prepared us for the disheartening reality that human aging is most likely a highly multifactorial phenomenon (Holliday, 2006; Kirkwood and Austad, 2000). Living things are not built to last forever but only (to a first approximation) long enough to reproduce. The ‘design lifetime’ of the soma places a similar durability requirement on its component parts, even if a few outperform this requirement due to innate stability. The available data suggests that the HSC pool is one of those subsystems whose functional life substantially exceeds the demands of the organismal lifespan. The device of setting aside a small number of multipotent cells for long-term tissue renewal recalls the way in which a set-aside of germline cells supports unlimited genomic and phenotypic renewal across generations. Even though sustenance of blood renewal capacity in old age is under weak selection pressure, it may be relevant that the genetic tool kit which has evolved for perpetual germline maintenance is at hand for stem cell maintenance. Owing to the small numbers of stem cells which must be conserved, and the fact that they have no effector duties, the energetic costs associated with anti-aging stratagems such as quiescence and apoptotic selection is presumably low relative to the situation in the differentiated cell types which make up bulk tissue.

Admittedly, stem cells are not exempted from the problem of antagonistic pleiotropy, as there is a tension between limitless self-renewal capacity and oncogenic potential. This theoretical consideration has encouraged the notion that HSC self-renewal is checked by telomere loss or aggressively thresholded apoptosis or senescence pathways. In light of the evidence against HSC exhaustion with age, more attention should be paid to the ways in which genome defense can stave off both cellular aging and malignancy. It may be that the surplus HSC renewal capacity found in mouse models is merely an unselected side benefit of the repair and quality control activity needed to ward against oncogenesis during the reproductive lifespan.

Certainly, much remains to be discovered about the roles of DNA damage and genomic maintenance in HSC aging. It would also be premature to write off loss of replicative capacity as a significant factor in human hematopoietic aging when so much of the available evidence on this topic comes from mouse models. Nevertheless, epigenetics is emerging as a particularly intriguing new frontier for HSC aging research. Immunosenescence is probably the most important blood aging phenotype in terms of its impact on human longevity and quality of life. There is evidence that this phenomenon has roots in gene expression changes within the stem cell compartment, although a link to genomic maintenance cannot be excluded. It will be important to determine if age-related changes in the transcriptome are a response to cell-extrinsic factors peculiar to hematopoiesis, or if they reflect instabilities in epigenetic regulation which manifest with the passage of time and repeated rounds of cell division. If the latter, the implications for aging research could extend well beyond the hematopoietic system.

Can we ever hope to use stem cells to rejuvenate the blood? HSC transplantation is the only stem cell therapy which is already established in the clinic, but there are great hurdles to overcome before it could be applied to ameliorate a non-life-threatening condition such as age-related immune deficiency. The develop-
ment of cytokine or small-molecule-based therapies to counter the effects of HSC lineage skewing may be a more realistic goal in the short term. In any case, it is still uncertain to what extent interventions targeting stem cells or early progenitors could benefit the elderly. Immunoseneescence appears to be multi-factorial in origin, probably involving cell-intrinsic changes at the level of lymphoid precursors and effector cells, as well as stromal and hormonal influences (reviewed in Gruver et al., 2007; Hakim and Gress, 2007; Lintorn and Dorshkind, 2004). The relative importance of different causal factors in this process remains to be determined. Further, it seems possible that the decline in B and T cell production with age is at least partly adaptive in character. Once the immune system has encountered a sufficiently broad array of antigens, the trade-off between adding to the antigenic repertoire and increasing false-positive, autoimmune responses may favor a reduction in new lymphocyte production (Gruver et al., 2007). If this hypothesis is correct, a restoration of lymphopoiesis to youthful levels might prove counterproductive. Nonetheless, it has been suggested that a shift in the peripheral lymphocyte populations towards antigen-experienced memory cells and away from naïve cells drives the entire spectrum of age-related immunological dysfunction, including heightened auto-reactivity and excessive inflammation, as well as diminished protective responses (Hakim and Gress, 2007; Johnson and Cambier, 2004). Clearly, we will have to resolve these fundamental issues before research findings can be translated to the clinic. In the interim, the remarkable experimental tractability of the hematopoietic system ensures that it will remain an important model for advancing our understanding of stem cell biology and its relationship to the aging process.

References


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