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SHORT TAKE

Age-related changes in human hematopoietic stem/progenitor cells

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Summary

Adult stem cells are critical for maintaining cellular homeostasis throughout life, yet the effects of age on their regenerative capacity are poorly understood. All lymphoid and myeloid blood cell lineages are continuously generated from hematopoietic stem cells present in human bone marrow. With age, significant changes in the function and composition of mature blood cells are observed. In this study, we report that age-related changes also occur in the human hematopoietic stem cell compartment. We find that the proportion of multipotent $CD34^+CD38^-$ cells increases in the bone marrow of elderly (>70 years) individuals. $CD34^+CD38^+CD90^-CD45RA^{+/-}CD10^-$ and $CD34^+CD33^+$ myeloid progenitors persist at the same level in the bone marrow, while the frequency of early $CD34^+CD38^+CD90^-CD45RA^+CD10^+$ and committed $CD34^+CD19^+$ B-lymphoid progenitors decreases with age. In contrast to mice models of aging, transplantation experiments with immunodeficient NOD/SCID/IL-2Rγ null (NSG) mice showed that the frequency of NSG repopulating cells does not change significantly with age, and there is a decrease in myeloid lineage reconstitution. An age-related decrease in the capacity of $CD34^+$ cells to generate myeloid cells was also seen in colony-forming assays *in vitro*. Thus, with increasing age, human hematopoietic stem/progenitor cells undergo quantitative changes as well as functional modifications.

Key words: aging; B-lymphopoiesis; hematopoietic stem cells; myelopoiesis; xenotransplantation.

Introduction

Aging is accompanied by a decline in immune responses (for review see (Min *et al.*, 2005)) and a higher rate of unexplained anemia (Guralnik *et al.*, 2004). These age-related changes could be because of either a general decrease in bone marrow (BM) cellularity (Ogawa *et al.*, 2000; Muschler *et al.*, 2001) or impaired hematopoietic stem cell (HSC) function. Animal models have led to the generally held idea that with age, there is an increase in the number of primitive HSC with decreased repopulating capacity and biased myeloid/lymphoid potential (for review see (Waterstrat & Van Zant, 2009)). These results are based primarily on the study of C57BL/6 mice; however, strain specific differences have been reported as to the impact of age on HSC numbers (Chen *et al.*, 2000). Furthermore, in the rhesus monkey, a decrease in HSC is observed with age and unlike mouse models, aging results in a decrease in myelopoiesis, while lymphopoiesis remains intact (Lee *et al.*, 2005).

In humans, only fragmentary and contradictory information is available concerning age-related changes in HSC (Waterstrat *et al.*, 2008; Taraldsrud *et al.*, 2009; Beerman *et al.*, 2010). One study (Chatta *et al.*, 1993) suggested that the proportion of HSC and in particular myeloid progenitors in mononucleated cells was the same in young and elderly adults but the generation of mixed granulocyte–macrophage colonies decreased with age (Marley *et al.*, 1999). Some studies concerning B-lymphopoiesis indicate a decrease in B-cell progenitors in the BM with age (Rego *et al.*, 1998; McKenna *et al.*, 2001), while others found no change (Rossi *et al.*, 2003). Here, we report that although the number of phenotypic $CD34^+CD38^-$ multipotent progenitor cells in human BM increases with age, there is no change in stem cell repopulating activity. Furthermore, the capacity to generate B-cell progenitors and myeloid cells as measured by both *in vivo* and *in vitro* assays is impaired.

Results and discussion

To investigate the effects of aging on human HSC, we first performed a phenotypic analysis of BM of healthy individuals, between the ages of 14 and 92 years (n = 48). These experiments showed that while the proportion of total BM $CD34^+$ cells remained stable with age, there was a significant increase in

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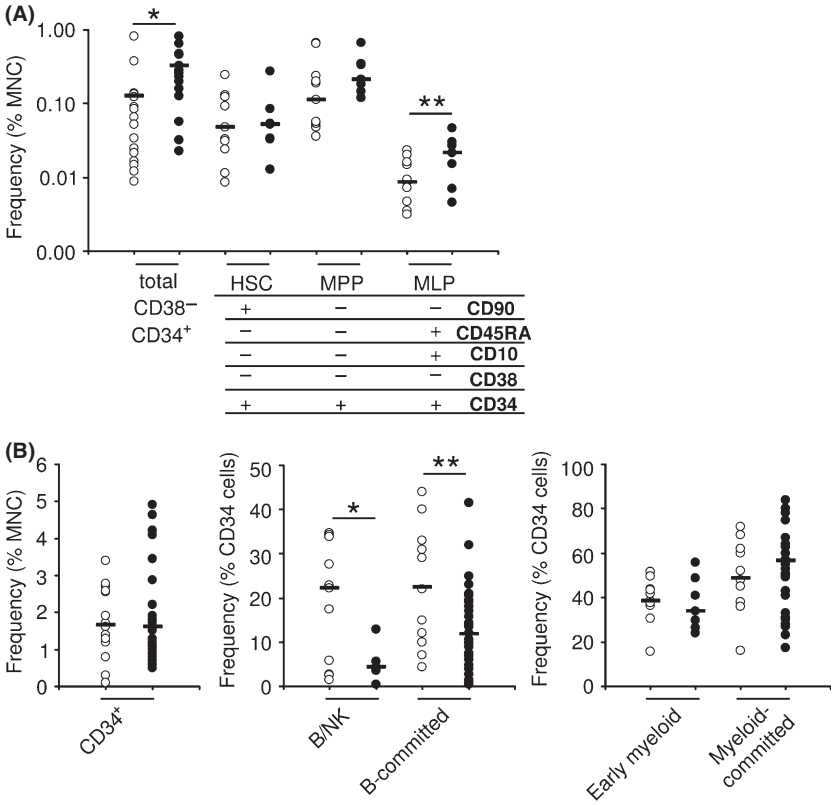

Fig. 1 Age-related changes in the frequency of hematopoietic progenitors in human bone marrow (BM). (A) Frequency of hematopoietic stem cell (HSC), multipotent progenitors (MPP) and multilymphoid progenitors (MLP) in BM mononuclear cells (MNC) isolated from young (< 40 year old; open circles) and elderly (> 70 year old; closed circles) individuals. *$P < 0.006$; **$P < 0.005$; Mann–Whitney test. Bar = mean. Phenotype of cell populations and isolation procedure are shown below the graph and in Fig. S3. (B) Frequency of committed progenitors in BM MNC. B/NK ($CD34^+CD38^+CD90^-CD45RA^+CD10^+$ cells); B-committed ($CD34^+CD19^+$ cells); early myeloid ($CD34^+CD38^+CD90^-CD45RA^{+/-}CD10^-$) and committed myeloid ($CD34^+CD33^+$) progenitors. *$P < 0.02$; **$P < 0.01$; Mann–Whitney test. Bar = mean.

the number of multipotent $CD34^+CD38^-$ cells (Fig. 1). Early ($CD34^+CD38^+CD90^-CD45RA^{+/-}CD10^-$) and committed ($CD34^+CD33^+$) myeloid progenitors persisted at the same level; however, the frequency of B/NK precursors ($CD34^+CD38^+CD90^-CD45RA^+CD10^+$) and committed B-lymphoid progenitors ($CD34^+CD19^+$) decreases with age (Fig. 1B). Notably, total $CD19^+$ cells in BM of elderly individuals returned to levels observed in young BM (Fig. S1).

We next compared the repopulating capacity of $CD34^+$ cells isolated from young (15–29 year, $n = 6$), middle aged (49–68 year, $n = 6$), and elderly (70–92 year, $n = 4$) individuals after injection of 10^5 cells into NOD/SCID/interleukin-2 receptor γ chain–null (NSG) mice. Twelve weeks after injection, all mice were engrafted, and the level of human chimerism was similar for all age-groups (Table S1). Limiting doses of cells (0.5×10^5, 0.1×10^5, and 0.05×10^5 cells) from young (14–25 year, $n = 3$) and elderly (83–87 year, $n = 3$) individuals (Table S2) showed no significant differences in either the level of human chimerism (Fig. 2A) or the frequency of NSG repopulating cells (Fig. 2B). However, $CD34^+$ cells from elderly individuals generated different proportions of myeloid and B-lymphoid cells in murine BM. We found a 5-fold decrease in myeloid cells, while B-lineage reconstitution remained the same (Fig. 2C). Colony-forming assays on methylcellulose confirmed the decrease in the capacity to generate various myeloid cells with age (Fig. 2D and Fig. S2). Analysis of B-lymphopoiesis by limiting dilution assays on MS-5 stromal cells showed a decrease in the frequency of B-cell progenitors in $CD34^+$ cells from elderly individuals (Fig. 2E) and maintained capacity to generate B-cells (Fig. 2F), in agreement with the phenotypic BM analyses (Fig. 1B and S1).

Collectively, our results indicate that the increased frequency of phenotypic $CD34^+CD38^-$ multipotent progenitor cells in human BM with age is not associated with increased NSG repopulating activity, but with a decreased capacity to generate B-cell progenitors and myeloid lineage cells. While further experiments are necessary to understand the mechanisms underlying these observations, this study provides evidence of quantitative and qualitative age-related changes in human hematopoietic stem/progenitor populations.

Experimental procedures

Cells

BM cells were isolated from young (10–37 year; mean = 23 year; $n = 24$; males 14/females 10) HSC allograft donors or

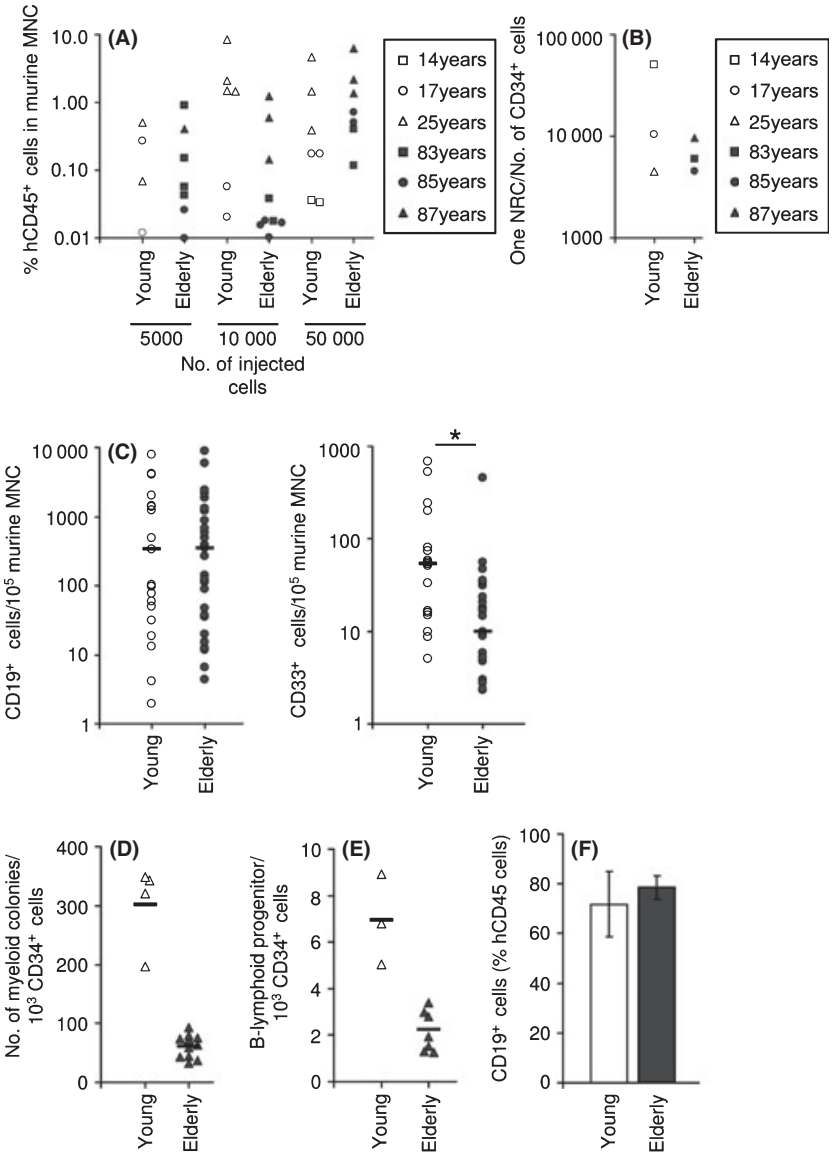

Fig. 2 Effects of age on myeloid and B-lymphoid potential of CD34$^+$ stem/progenitor cells. Multilineage engraftment (A), frequency of NOD/SCID/IL-2Rγ null (NSG) repopulating cells (B) and lineage-specific reconstitution in NSG mice (C). Limiting doses (0.5×10^5, 0.1×10^5 and 0.05×10^5) of CD34$^+$ cells from young (14–25 year; $n = 3$) and elderly (83–87 year; $n = 3$) individuals were injected intravenously into NSG mice (for details see Table S2 and Fig. S4). Twelve weeks after injections, human chimerism in murine bone marrow (BM) was measured by FC analysis and the frequency of NSG repopulating cells (NRC) was calculated using L-Calc software; *$P < 0.01$; Mann–Whitney Test. (D) The capacity of CD34$^+$ cells isolated from young (10–37 year, mean = 26 year, $n = 4$) and elderly (71–89 year, mean = 77 year, $n = 11$) individuals to generate myeloid colonies was determined by *in vitro* methylcellulose assays and is significantly different $P < 0.006$; Student's *t*-test. Bar = mean. (E) The frequency of B-lymphoid progenitors in CD34$^+$ cells isolated from young (10–37 year, mean = 21 year, $n = 3$) and elderly (71–89 year, mean = 77 year, $n = 7$) individuals was determined by *in vitro* limiting dilution assay on MS-5 stromal cells and was significantly different $P < 0.04$; Student's *t*-test. Bar = mean. (F) The capacity of CD34$^+$ cells isolated from young (10–37 year; mean = 21 year; $n = 3$) and elderly (71–89 year; mean = 77 year; $n = 7$) individuals to generate B-cells *in vitro* was measured as the percent of CD34$^-$CD19$^+$ cells in hCD45$^+$ cells after 4-week culture. Histograms are means ± SD.

from middle age (49–68 year; mean = 57 year; $n = 6$; males 4/females 2) and elderly (69–92 year; mean = 78 year; $n = 55$; males 16/females 39) individuals free of hematopoietic or inflammatory disease undergoing hip replacement. Donors signed informed consent, and this study has been approved by the IRB of Paris North Hospitals. Mononuclear cells (MNC) were harvested using a Ficoll gradient. CD34$^+$ cells were isolated with CD34 MicroBead kit (Milenyi Biotec, Bergish Gladbach, Germany) according to the manufacturer's protocol.

Phenotyping

Stainings were performed using antibodies from Becton Dickinson as described in Fig. S3 for cytofluorometric analysis of hematopoietic progenitors in human bone marrow (Doulatov et al., 2010) and in Fig. S4 for analysis of human chimerism in xenotransplantation experiments. Flow cytometry (FC) was performed using BD-FACS Canto II, and results were analyzed using Diva Software 6.1.2.

In vitro assays

To measure the generation of myeloid colonies, $CD34^+$ cells (2500 cells per mL) were seeded in methylcellulose medium (HSC005; R&D Systems, Lille, France) and colonies were counted after 12 days. Cultures supporting B-lymphopoiesis were performed in 96-well plates on a monolayer of MS-5 cells in RPMI1640-GlutaMax medium (Gibco, Cergy Pontoise, France) with 10% fetal bovine serum (HyClone) without added cytokines (Nishihara *et al.*, 1998). The capacity of $CD34^+$ cells to generate B-cells was measured as the percentage of $CD34^-CD19^+$ cells among $hCD45^+$ cells after 4 weeks in cultures seeded with 100 cells. To measure the frequency of B-lymphoid progenitors, limiting dilutions of $CD34^+$ cells (250, 150, 100, 50 cells per well) were performed. Frequencies were estimated using L-Calc (StemSoft Software, Vancouver BC, Canada) based on the numbers of wells containing human B-cells ($CD45^+CD19^+CD33^-CD34^-$). Forty-eight wells were analyzed per cell concentration.

Xenotransplantation

Eight-week-old NOD/SCID/interleukin-2 receptor γ chain–null mice (Shultz *et al.*, 2005) (Jackson Laboratories, Kent, England) were housed at CRUK and Institute Paoli-Calmettes animal care facilities in accordance with institutional guidelines. Sublethally irradiated animals were injected intravenously with $hCD34^+$ cells. Twelve-week posttransplant engraftment in murine BM was assessed by FC analysis. Threshold engraftment was set at 0.01% of $hCD45^+$ cells comprising at least 1% of both $CD19^+$ and $CD33^+$ cells. Frequency of NSG repopulating cells was estimated based on limiting dilution assays using L-Calc (StemSoft Software).

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Disclosure of conflicts of interest

The authors declare no competing financial interests.

Author contributions

Klaudia Kuranda was involved in manuscript writing, conception and design, data analysis and interpretation, collection and assembly of data. Jacques Vargaftig, Philippe de la Rochere and Florence Bardin assisted with the collection of data. Dominique Charron was the head of department and supported the study. Christine Dosquet was involved in the final approval of manuscript. Cecile Tonnelle provided the study material and was involved in data interpretation. Dominique Bonnet assisted with the provision of study material, data analysis and interpretation, collection of data, final approval of manuscript. Michele Goodhardt was involved in the conception and design, data interpretation and manuscript writing.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1 Engraftment efficiency in NSG mice of non-limiting doses of CD34$^+$ cells (10^5 cells per mouse) with age.

Table S2 Limiting dilution assays with CD34$^+$ cells from young and elderly individuals in NSG mice.

Fig. S1 Frequency of CD19$^+$ cells in human BM with age.

Fig. S2 Decreased generation of myeloid colonies from CD34$^+$ cells with age.

Fig. S3 Cytofluorometric analysis of hematopoietic progenitors in human BM.

Fig. S4 Cytofluorometric analysis of B-lymphoid and myeloid lineage reconstitution in BM of NSG mice.

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