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Aging Impairs Long-Term Hematopoietic Regeneration after Autologous Stem Cell Transplantation

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A B S T R A C T

Most of our knowledge of the effects of aging on the hematopoietic system comes from studies in animal models. In this study, to explore potential effects of aging on human hematopoietic stem and progenitor cells (HSPCs), we evaluated $CD34^+$ cells derived from young (<35 years) and old (>60 years) adult bone marrow with respect to phenotype and in vitro function. We observed an increased frequency of phenotypically defined stem and progenitor cells with age, but no distinct differences with respect to in vitro functional capacity. Given that regeneration of peripheral blood counts can serve as a functional readout of HSPCs, we compared various peripheral blood parameters between younger patients (≤50 years; n = 64) and older patients (≥60 years; n = 55) after autologous stem cell transplantation. Patient age did not affect the number of apheresis cycles or the amount of $CD34^+$ cells harvested. Parameters for short-term regeneration did not differ significantly between the younger and older patients; however, complete recovery of all 3 blood lineages at 1 year after transplantation was strongly affected by advanced age, occurring in only 29% of the older patients, compared with 56% of the younger patients ($P = .009$). Collectively, these data suggest that aging has only limited effects on $CD34^+$ HSPCs under steady-state conditions, but can be important under consitions of chemotoxic and replicative stress.

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INTRODUCTION

The bone marrow (BM) is one of the body's most highly self-renewing tissues. Hematopoietic stem cells (HSCs) are capable of replenishing all blood cell types during the complete lifespan of an organism. The hematopoietic system does not escape the effects of aging, however. In humans, these effects are manifested clinically by an increased incidence of myeloproliferative diseases, including leukemia [1-3], decreased adaptive immunity [4-6], and increased propensity for anemia [7,8]. Experimental studies in the murine system comparing young and old HSCs have demonstrated skewing toward a more myeloid-biased output [9-11], a relative increase in phenotypically defined HSCs [10-14], and diminished competitive repopulating ability of old murine HSCs [10-12].

Until recently, data on age-associated changes in human hematopoietic stem and progenitor cells (HSPCs) were limited. Two recent studies that addressed this issue suggested that, although some of the findings observed in the murine system could be confirmed in humans, increasing age seems to have only a limited impact on the phenotype and functional in vitro and in vivo properties (as examined in xenotransplant models) of human HSPCs [15,16].

In this study, we investigated $CD34^+$ cells derived from normal BM (NBM) of healthy young and elderly individuals with respect to phenotype and in vitro function. To study the impact of aging and stress response on human HSPCs in vivo, we analyzed the impact of age on HSPC mobilization and short-term and long-term regeneration after autologous stem cell transplantation (ASCT). A better understanding of HSPC aging will facilitate the development of more suitable treatment regimens for elderly patients undergoing ASCT.

MATERIALS AND METHODS

NBM

After achieving informed consent, BM aspirates were obtained from patients age >60 years who underwent total hip replacement, volunteers age 18 to 35 years, and young healthy potential donors for hematopoietic ASCT who underwent BM aspiration as part of a standard medical examination. NBM from 9 young healthy volunteers and 2 young potential donors was used for in vitro experiments. Young NBM samples for the microarray analysis came from 9 young healthy volunteers and 1 young potential donor. All older NBM samples came from patients undergoing total hip replacement. The protocol for NBM collection was approved by the Institutional Review Board of the University Medical Center Groningen. All participants had normal general health and normal peripheral blood counts and an absence of hematologic disorders. Details of the experiments performed on the NBM samples are presented in Supplemental Table 1.

Flow Cytometry Analysis and Sorting Procedures

The flow cytometry and sorting procedures are described in detail in the Supplemental Materials.

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In Vitro Culture Assays

A colony-forming cell (CFC) assay was performed in methylcellulose (MethoCult H4230; StemCell Technologies, Grenoble, France), supplemented with 20 ng/mL IL-3, 20 ng/mL IL-6, 20 ng/mL granulocyte colony-stimulating factor (G-CSF), 20 ng/mL c-Kit ligand, and 1 U/mL erythropoetin (Cilag Eprex, Brussels, Belgium), as described previously [17]. The assay was performed in duplicate. At 2 weeks after plating, colony-forming unit–granulocyte/macrophage (CFU-GM), burst-forming unit–erythroid (BFU-E), and colony-forming unit–granulocyte erythrocyte monocyte megakaryocyte (CFU-GEMM) numbers were measured and recorded.

For the long-term cultures, $CD34^+$ cells were plated in bulk in 12-well plates or, for limiting dilution experiments, in a 96-well plate precoated with MS5 stromal cells. Cells were expanded in long-term culture (LTC) medium (α-minimum essential medium supplemented with heat-inactivated 12.5% FCS; Sigma-Aldrich, Zwijndrecht, The Netherlands), heat-inactivated 12.5% horse serum (Sigma-Aldrich), penicillin and streptomycin, 2 mM glutamine, 57.2 μM β-mercaptoethanol (Sigma-Aldrich), and 1 μM hydrocortisone (Sigma-Aldrich), supplemented with 20 ng/mL IL-3, 20 ng/mL G-CSF (Rhone-Poulenc Rorer, Amstelveen, The Netherlands), and 20 ng/mL thrombopoietin (Kirin, Tokyo, Japan). Cultures were maintained at 37 °C in 5% CO_2 and were demi-depopulated weekly for medium changes. In the MS5 cocultures, cells were counted weekly.

For the long-term culture initiating cell (LTC-IC) limiting dilution assay, 5 weeks of expansion was followed by the removal of suspension cells and addition of methylcellulose (StemCell Technologies) as described for the CFC assay. Two weeks later, wells containing CFCs were scored as positive, and the LTC-IC frequency was calculated using L-Calc software for limiting dilution analysis (StemCell Technologies). Liquid cultures were performed exactly as described for the cocultures, but without coculturing of MS5.

Transplantation Procedure

Details on the apheresis and transplantation procedures are provided in the Supplemental Material.

Clinical Cohort of ASCT Recipients

A cohort of 119 ASCT recipients was retrospectively studied. All subsequent patients with multiple myeloma (MM) or non-Hodgkin lymphoma (NHL) age ≤50 years or ≥60 years treated with high-dose chemotherapy followed by ASCT at the Department of Hematology, University Medical Center Groningen between June 1993 and June 2007 were included in this study. Patients who experienced progressive or relapsing disease within 1 year after ASCT were excluded from the analysis. Within the NHL group, only patients with diffuse large B cell lymphoma or mantle cell lymphoma were included. The younger age group (≤50 years) comprised 64 patients (mean age, 44 ± 7 years), including 27 with NHL and 37 with MM. The older age group (≥60 years) included 55 patients (mean age, 63 ± 2 years), with 18 with NHL and 37 with MM. All patients with MM underwent ASCT upfront after induction therapy with vincristine, adriamycin, and dexamethasone (VAD) or with thalidomide, adriamycin, and dexamethasone (TAD) [18-20]. All patients with NHL underwent ASCT as second-line treatment, after first-line treatment with 6 to 8 cycles of (Rituximab), Cyclophosphamide, Hydroxydaunorubicin, Vincristine (Oncovin), Prednisone [(R)-CHOP] chemotherapy. Reinduction treatment consisted of (R) Cisplatin, Cytarabine, Dexamethasone [(R)-DHAP] followed by (R) Etoposide, Ifosfamide, Methotrexaat (R)-VIM and a second course (R)-DHAP chemotherapy [21]. Patients with MM were conditioned with high-dose melphalan (200 mg/m^2) (n = 70), cyclophosphamide + total body irradiation (n = 2), or Carmustine, Etoposide, Cytarabine, Melphalan (BEAM) (n = 2). Patients with NHL were conditioned with BEAM (n = 45).

After infusion of peripheral blood stem cells (PBSCs), the number of days needed to reach a granulocyte count of at least 0.5×10^9/L and to reach a thrombocyte count of at least 20×10^9/L was recorded for each patient. Moreover, cell counts were measured on day 28, day 40, and 1 year after ASCT. A leukocyte count of $\geq 4 \times 10^9$/L, thrombocyte count of $\geq 150 \times 10^9$/L, and hemoglobin (Hb) level of ≥7.5 mmol/L for women and ≥8.7 mmol/L for men were considered normal values in this analysis.

Statistical Analysis

The Mann-Whitney *U* test or χ^2 test was used for analysis of individual group differences. Hematologic recovery was assessed using Kaplan-Meier probability curves, and statistical comparison of curves was performed using the log-rank test. The prognostic value of different variables for probability of hematologic recovery was assessed by univariate and multivariate analyses using a Cox multiple regression model. Differences with a *P* value ≤.05 were considered statistically significant.

RESULTS

Age-Associated Phenotypic Changes Within the Human Hematopoietic System

To investigate whether the aging process has an effect on the composition of the different stem and progenitor cells populations within the total cell population, we performed phenotypic analyses of the total mononuclear cell fraction in young (age <35 years) and old (age >60 years) adult NBM. The percentages of total $CD34^+$ cells, progenitor cell–containing $CD34^+CD38^+$ cells, and multipotent $CD34^+CD38^-$ fractions increased significantly from young NBM (n = 5) to old NBM (n = 8) (Figure 1A). Subsequent analysis of the frequency of common myeloid progenitors, granulocyte macrophage progenitors, and megakaryocyte erythrocyte progenitors within the $CD34^+CD38^+$ fraction revealed no significant differences between young NBM (n = 4) and old NBM (n = 4) (Figure 1B). In contrast, the percentage of lymphoid progenitors, defined as $CD34^+CD10^+$ cells within the total $CD34^+$ cell population, was significantly lower in old NBM ($P = .021$) (Figure 1C).

Impact of Aging on HSPC Function In Vitro

We performed several in vitro assays to study the functional capacity of $CD34^+$ cells derived from young and old adult NBM. The colony-forming potential of $CD34^+$ cells for all included lineages was comparable in young NBM (n = 6) and old NBM (n = 5) (Figure 1D). In addition, the LTC-IC frequency, an established in vitro measurement for HSPCs, was comparable in young and old adult NBM-derived $CD34^+$ cells (Figure 1E).

To further assess the impact of age on function and differentiation potential of $CD34^+$ human cells, we cultured the cells in liquid and MS5 cocultures. As in the other in vitro assays, we found no significant difference in growth between $CD34^+$ cells derived from young adult NBM (n = 4) and those derived from old adult NBM (n = 4) (Figure 1F). In addition, we observed no differences in serial replating capacity assessed in the MS5 cocultures between young NBM and old NBM (data not shown). Taken together, these findings indicate very similar in vitro functional capacities of young and old adult NBM, as measured in the different in vitro assays.

Our observation that young and old NBM $CD34^+$ cells are quite comparable in steady-state conditions was confirmed by the results of our microarray-based gene expression analyses of $CD34^+$ cells derived from young NBM (n = 10) and old NBM (n = 22). We found a very limited number of differentially expressed genes, with 18 up-regulated probes representing 14 annotated genes ($P < .00001$, Student *t* test) (Supplemental Table 2).

HSPCs In Vivo

To follow up on our in vitro research on the impact of aging on human HSPC function, we extended our research to an in vivo situation, studying ASCT. In this setting, we studied mobilization and apheresis of PBSCs and compared regeneration data in a group of 64 younger patients (age ≤50 years) and a group of 55 older patients (age ≥60 years) after ASCT. Patients with progressive or relapsing disease within 1 year after ASCT were excluded from our analysis. Patient characteristics are summarized in Table 1.

Mobilization and Apheresis

Different mobilizing therapy regimens were used, with the percentages of patients receiving any 1 of these therapies not differing significantly in the 2 age groups (Table 1). The target for cell harvest was 5×10^6 $CD34^+$ cells/kg for patients

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