BIOLOGY

Hematopoietic Stem Cells Survive Circulation Arrest and Reconstitute Hematopoiesis in Myeloablated Mice

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Hematopoietic stem and progenitor cells (HSPC) for bone marrow transplantation are currently obtained directly from living voluntary donors or from cord blood units. However, a suitable donor is not always found. Because HSPC are known for their relative resistance to hypoxia, using an experimental murine model, we explored cadaveric bone marrow (BM) as their alternative source. After donor mice were sacrificed, BM was left in intact femurs at 37°C, 20°C, or 4°C under ischemic conditions, resulting in combined oxygen and metabolic substrate shortage and the accumulation of metabolic waste products. BM cells were harvested after a set time period ranging from 0 to 48 hours. To determine the impact of delayed harvesting on the transplantability of HSPC, a competitive repopulation assay using a murine Ly5.1/Ly5.2 congenic model in 2 different settings was used: after submyeloablative (6 Gy) or myeloablative (9 Gy) total-body irradiation, Ly5.2 hosts received cadaveric Ly5.1 cells or a mixture of cadaveric Ly5.1 cells and fresh Ly5.2 cells in a 1:1 ratio. Chimerism resulting from cadaveric donor cells, followed up to 6 months after transplantation, proved that the long-term repopulation ability of HSPC was fully preserved for 2 hours, 6 hours, and 12 hours at 37°C, 20°C, and 4°C of ischemia, respectively. A colony-forming unit-spleen (CFU-S) clonogenic assay revealed a higher sensitivity of proliferating hematopoietic progenitors to ischemia compared to repopulating cells (STRC and LTRC). Flow cytometry analysis of apoptosis in cadaveric BM demonstrated that the LSK ($Lin^{low}Sca\text{-}1^{+}c\text{-}Kit^{+}$) subpopulation, enriched in HSPC, contained less apoptotic and dead cells than the BM as a whole. Furthermore, the number of LSK SLAM ($CD150^{+}CD48^{-}$) and LSK SP (side population) cells (fractions highly enriched in hematopoietic stem cells) decreased in parallel with BM transplantability. As well as cadaveric BM cells, we also tested the transplantability and survival of BM cells after storage in a suspension in vitro without specific hematopoietic growth factors. HSPC did not display any decrease in transplantability after 2 days of storage at 37°C or 4 days at 4°C. A higher sensitivity of progenitors to unfavorable conditions was observed again using CFU-S and granulocyte macrophage-colony forming cell (GM-CFC) assays, especially at 37°C. This paper shows that HSPC survive the cessation of circulation for a considerable time and maintain their engraftment potential. This time is significantly extended with in vitro storage compared to the cadaveric BM.

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INTRODUCTION

Bone marrow transplantation (BMT) is a widely used therapeutic method for the treatment of hematologic disorders, solid tumors, or autoimmunity diseases. During the transplantation procedure, hematopoietic stem and progenitor cells (HSPC) obtained from the BM of living donors are transplanted to myeloablated patients. The HSPC donor is usually a relative of the patient or an HLA-matched volunteer from a registry [1]. Another source of HSPC is umbilical cord blood, obtained during childbirth and preserved in a cord blood unit [2]. Despite the international cooperation between donor registries, in some cases suitable HSPC for transplantation are not found because the patient has an unusual HLA-phenotype [1]. Therefore, finding new ways to enlarge the number of donors remains a challenging task.

As was mentioned above, at present HSPC for transplantation are harvested exclusively from living donors or their source is cord blood. Nevertheless,

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Soderdahl et al. [3] explored the BM from cadaveric organ donors and based on cell viability and colony-forming unit (CFU) numbers, they assumed that the cells could be procured with a high degree of engraftment potential. In addition, a CD34-positive cell fraction was not affected by storage for up to 3 days in a heparinized RPMI 1640 medium at room temperature (20°C) or in a refrigerator (4°C-8°C) [3]. Furthermore, BM cells harvested from cadaveric organ donors and stored at 4°C for 7 days did not display a significant increase in apoptosis [4].

Organs for transplantation are routinely harvested from non-heart-beating donors (NHBD) [5-7]. Four categories of NHBD are identified: I (dead on arrival) and II (unsuccessful resuscitation) are uncontrolled donors; III (withdrawal of life-supporting therapy) and IV (cardiac arrest in a brain-dead donor) are controlled donors [8]. Whereas the transplantability of solid organs from NHBD and the tolerance of the organs to warm ischemia has been widely studied over the last two decades [5-7,9-11], practically no attention has been paid to the BM, despite the fact that hematopoietic stem cells, responsible for long-term repopulation, reside in a hypoxic microenvironment of the BM, stem cell niches [12,13]. Hypoxia, together with the cytokines produced by the niche keep hematopoietic stem cells quiescent [14-16], and this could favor their survival after the cessation of circulation.

It can be assumed that these properties also enable HSPC to survive in vitro, for instance, when they are stored in a medium. Although the cells should be used for BMT as soon as possible, sometimes this liquid storage lasts for several hours or even days [17]. This can be because of harvesting being done via consecutive leukapheresis to collect a sufficient amount of cells or because the cells need to be transported to the patient, for example, to another country. The most commonly used temperature for the storage of BM cells or peripheral blood progenitor cells in a suspension is 4°C to 8°C, with the recommendation to not exceed 20°C, and cells are usually transplanted within 72 hours after their initial collection.

In this study, we focused on the tolerance of murine HSPC, residing in their niches, to interruption of the blood supply leading to anoxia and metabolic starvation. The survival and transplantability of HSPC collected from such cadaveric BM was compared to those maintained in a tissue culture medium. HSPC were analyzed by phenotyping, by clonal assays and by competitive repopulation assay.

MATERIALS AND METHODS

Animals

C57BL/6 mice (Ly5.2 and Ly5.1) were maintained in a clean conventional animal facility with a light-dark cycle of 12 hours and fed ad libitum. Eight- to 12-week-old mice were used in the experiments. All experiments were approved by the Laboratory Animal Care and Use Committee of the First Faculty of Medicine, Charles University, and were performed in accordance with national and international guidelines for laboratory animal care.

Cadaveric BM

Donor mice were sacrificed by cervical dislocation. Intact femurs were removed and kept in preheated/precooled phosphate-buffered saline (PBS) at 37°C, 20°C, and 4°C, respectively, for various time periods up to 48 hours. Subsequently, the ends of the femur were opened and BM cells ("cadaveric BM") were flushed into a PBS with 0.5% bovine serum albumin (BSA) solution (PBS/BSA further on) and kept on ice. A single-cell suspension was prepared and the number of nucleated cells was determined using an AUTO T4 Cellometer (Nexelom Bioscience, Lawrence, MA). Afterward, the survival and transplantability of cadaveric BM cells was determined.

In Vitro Storage of BM Cells

Briefly, BM cells were harvested from the femurs of sacrificed C57Bl/6 donors. Afterward, cells were washed and resuspended in IMDM medium (Lonza Biologics Inc., Portsmouth, NH) with 10% fetal calf serum (FCS; Sigma Aldrich Corp., St. Louis, MO) at a concentration of 10×10^6 cells/mL. Cells were maintained in the medium for up to 10 days at 37°C or 4°C, respectively. Subsequently, the survival of hematopoietic progenitor cells was analyzed via the granulocyte-macrophage-colony forming cells (GM-CFC) cultivation method and colony-forming units-spleen (CFU-S) method. Intervals of 1 and 2 days at 37°C and 2 and 4 days at 4°C were also used for a competitive transplantation repopulation assay.

Flow Cytometry Analysis of LSK SLAM and LSK SP Cells

Fresh and cadaveric BM was analyzed by flow cytometry for subpopulations enriched in stem cells—the LSK SLAM [18] ($Lin^{low}Sca\text{-}1^{+}c\text{-}Kit^{+}CD150^{+}CD48^{-}$) and LSK SP ($Lin^{low}Sca\text{-}1^{+}c\text{-}Kit^{+}$, side population, Hoechst 33342 negative). The SP population was stained according to Goodell et al. [19]. Briefly, cells were resuspended in preheated IMDM medium ($1\text{-}2 \times 10^6$ cells per 1 mL of IMDM). Hoechst 33342 (Honeywell Riedel-de Haën, Seezle, Germany) was added to a final concentration of 5 μg/mL and the suspension was incubated for exactly 90 minutes at 37°C. Afterward, the cells were washed with ice-cold PBS/BSA and stained for specific surface markers of LSK and SLAM cells with fluorescein-conjugated monoclonal antibodies (20 min on ice and in the

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