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Lack of Correlation between an Assay Used to Determine Early Marrow Allograft Rejection and Long-Term Chimerism after Murine Allogeneic Bone Marrow Transplantation: Effects of Marrow Dose

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ABSTRACT

The acute rejection of bone marrow (BM) allografts by host effectors can occur within a short period after BM transplantation (BMT) in lethally irradiated mice. Common assays used to ascertain engraftment/resistance involve measuring the growth of granulocyte/monocyte progenitors (colony-forming unit–granulocyte-macrophage) in vitro or splenocyte proliferation assessed by radioisotope incorporation in vivo 5 to 8 days after BMT. However, the correlation of the long-term outcome of BMT with the kinetics of recovery by using the dose of allogeneic BM cells (BMCs) that leads to early rejection as determined by the in vitro assessment has not been extensively studied. Thus, to investigate whether the early rejection of donor BMCs is an indication of a long-term engraftment failure, C57BL/6 ($H2^b$) mice were lethally irradiated and transplanted with various doses of BALB/c ($H2^d$) BMCs. The short-term engraftment of donor precursors (colony-forming unit–granulocyte-macrophage), the kinetics of hematopoietic cell recovery, the extent of donor chimerism, and the proportion of the recipients with long-term survival were determined. The results show that the kinetics and extent of hematopoietic cell recovery were significantly delayed in mice receiving limiting doses of BMCs that were rejected or severely resisted at day 8 after BMT. However, a proportion of these mice survived up to 98 days after BMT with mixed chimerism or donor chimerism. This study demonstrates that early rejection of BM precursors, as assessed by measurement of myeloid progenitors in the spleen after BMT, does not always correlate with the long-term outcome of the marrow allograft and that significant variability is inherent in the extent of chimerism when threshold amounts of BMCs are used.

KEY WORDS

Acute rejection • Bone marrow transplantation • Long-term chimerism • CFU-GM

INTRODUCTION

Bone marrow transplantation (BMT) or stem cell transplantation has been increasingly used in recent years as a treatment for neoplastic and nonneoplastic diseases of hematologic or nonhematologic origin [1-5]. Because of the scarcity of related HLA-matched donors, bone marrow (BM) allografts from related HLA-mismatched or HLA-matched unrelated donors are used in allogeneic BMT. However, the efficacy of allogeneic BMT is challenged by serious obstacles, most of which are fatal. These obstacles include the failure of allograft engraftment, graft-versus-host disease (GVHD) mediated by donor T cells contaminating the BM, relapse from the original tumor when used as a cancer therapy, and opportunistic infection due to severe immunosuppression resulting from the conditioning regimen for BMT [2,3,5,6]. Resolving one problem without worsening another poses a considerable challenge and contributes to the complexity of allogeneic BMT. For example, depletion of T cells from the BM allograft to minimize

GVHD increases the incidence of engraftment failure of donor cells because of the concurrent removal of engraftment-facilitating T cells [7-11].

Over the past couple of decades, the phenomenon of BM cell (BMC) rejection/engraftment has been actively investigated. Previous studies have shown that natural killer (NK) cells can reject BMC allografts in vivo [12-15] and that the subsets of NK cells may play different roles in BMC rejection in mouse models of BMT [15-17]. However, the cell populations targeted in BM allograft rejection are not clearly understood. Despite many years of research, an in vitro assay that adequately reflects the immunogenetics of BM allograft rejection in vivo is currently unavailable, and the in vivo assays have remained unchanged. In these in vivo assays, mice are given myeloablative conditioning, usually in the form of total body irradiation at various doses depending on the strain of mice, and then infused with BMCs intravenously [18]. At early time points within 2 weeks after BMT, the level of BMC engraftment is determined by measuring the hematopoietic or granulocyte/monocyte progenitor cell content (colony-forming unit–culture and colony-forming unit–granulocyte-macrophage [CFU-GM]) in spleen or splenic incorporation of a radiolabeled thymidine analog, ^{125}I-iododeoxyuridine [14,18]. In previous studies using the in vivo assays, long-term donor cell engraftment has not been assessed. Only in studies with experimental designs focused on long-term donor chimerism has the percentage of donor cell chimerism been determined [19,20]. Furthermore, a direct comparison of allogeneic BMT outcomes (early rejection/engraftment, kinetics of hematopoietic cell recovery, and long-term donor chimerism) using various doses of allogeneic BMCs, including one that results in early rejection, has not yet been performed. Therefore, we investigated whether early rejection of the BM allograft as indicated by a currently used assay is reflective of delayed or failed hematopoietic cell recovery and long-term engraftment of donor BMCs. In this report, we demonstrate that (1) an early lack of growth of donor BMCs as measured by CFU-GM is reflected in delayed hematopoietic cell recovery and (2) the lack of growth of donor BMCs as measured by CFU-GM does not always reflect the level of long-term donor cell engraftment.

MATERIALS AND METHODS

Mice

C57BL/6 (B6, H2^b) and BALB/c (H2^d) mice were purchased from Jackson Laboratory (Bar Harbor, ME) and the Animal Production Area (National Cancer Institute, Frederick, MD), respectively. All mice were kept in a specific pathogen–free condition and used at 8 to 14 weeks of age.

Bone Marrow Transplantation

B6 mice were put on drinking water containing 2.5% bleach (vol/vol) for 7 days before and after BMT, and at day 0, B6 mice were lethally irradiated with a single exposure of γ irradiation from a ^{137}Cs source (2.12 Gy/min) at 9.5 Gy. A single-cell suspension of BMCs from BALB/c mice was prepared in Dulbecco's phosphate buffered saline and injected into the caudal vein of B6 recipients at 15×10^6, 5×10^6, or 2.5×10^6 per mouse (intravenously) 2 hours after irradiation. For the purpose of discussion, the BMC dose at 15×10^6, 5×10^6, or 2.5×10^6 is referred to as high, intermediate, or low, respectively, in the text. Mice were monitored for survival, and analyses determining the level of hematopoietic reconstitution were performed at various days after BMT, as described below.

Short-term Reconstitution Studies

At day 8 to 12 after BMT, mice transplanted with 2.5×10^6, 5×10^6, or 15×10^6 BALB/c BMCs were killed (3-5 mice per group per experiment), and a single-cell suspension of each spleen was prepared. Two hundred fifty thousand or 5×10^5 splenocytes were cultured in colony assay medium (Iscove's modified Dulbecco's medium containing 15% fetal bovine serum, 100 U/mL penicillin/100 μg/mL streptomycin, 2 mmol/L L-glutamine, 5×10^{-5} mol/L 2-mercaptoethanol, and 1.1% methylcellulose [wt/vol]) supplemented with 10 ng/mL recombinant murine (rm) granulocyte-macrophage colony-stimulating factor (GM-CSF; AMGen Corporation, Thousand Oakes, CA) and 10 ng/mL rm interleukin-3 (Developmental Therapeutics Program, National Cancer Institute) in a 35-mm petri dish in triplicate for 7 days in a humidified atmosphere at 37°C with 5% CO_2. Granulocyte/monocyte colonies were then enumerated on a stereo microscope (Nikon, Melville, NY).

Complete Blood Count Analysis

For assessing the kinetics of hematopoietic cell recovery, peripheral blood samples were collected weekly from 3 to 5 mice per group into ethylenediaminetetraacetic acid–treated Microtainer tubes (Becton Dickinson, San Jose, CA) for 6 weeks after BMT. The blood samples were analyzed on a HEMAVET Multispecies Hematology Analyzer (CDC Technologies, Oxford, CT) to determine the complete blood count (CBC) levels.

Antibodies

Fluorescein isothiocyanate (FITC)–conjugated anti-mouse H2D^d (FITC-H2D^d); phycoerythrin (PE)–conjugated anti-mouse H2K^b, CD4, and CD8 (PE-H2K^b, PE-CD4, and PE-CD8); biotinylated anti-mouse