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ScienceDirect
Immunobiology 213 (2008) 455–468
Immunobiology
www.elsevier.de/imbio

Facilitation of hematopoietic recovery by bone grafts with intra-bone marrow–bone marrow transplantation

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Received 5 June 2007; received in revised form 20 October 2007; accepted 26 October 2007

Abstract

We have previously shown that T cells can acquire donor-type major histocompatibility complex (MHC) restriction and can interact with both donor-type antigen-presenting cells (APCs) and B cells, when adult donor bones are co-grafted with intravenous (IV) injection of bone marrow cells (BMCs) in order to supply donor bone marrow (BM) stromal cells. We have also found that the direct injection of donor BMCs into recipient BM (intra-bone marrow–bone marrow transplantation: IBM–BMT) produces more rapid reconstitution (including T-cell functions) and higher survival rates than IV injection (IV-BMT) even in chimerism-resistant combinations. In the present study, we show that the co-administration of bones from suckling (2–3 days old) donor mice is also effective in the IBM–BMT system. Even when a relatively low number of BMCs were injected into adult (more than 15 weeks old) mice, complete reconstitution was achieved in the mice that had received IBM–BMT + bone grafts, but not in the mice that had received IBM–BMT alone. Most BM and splenic adherent cells obtained from the recipients that had received IBM–BMT + bone grafts were reconstituted by donor-type cells. Both T-cell proliferation and plaque-forming cell assays indicated that the T cells of such mice showed donor-type MHC restriction. Moreover, the analyses of thymic sections using confocal microscopy revealed that donor BM stromal cells had migrated into the thymus. Thus, the co-administration of donor bones has great advantages for allogeneic BMT in adult mice.

Keywords: Bone marrow transplantation; Bone marrow cells; Bone graft; Stromal cells

Introduction

Allogeneic bone marrow transplantation (BMT) has been utilized for complete treatments for refractory diseases such as leukemia and aplastic anemia. It has

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doi:10.1016/j.imbio.2007.10.015

been reported, however, that humoral immunodeficiency lasts for a longer period in adult patients than in infant patients even after donor-type cells are reconstituted (Onoe et al., 1980). Similar observations are obtained in animal studies; primary humoral responses against T cell-dependent antigens cannot be completely reconstituted in fully allogeneic BMT (El-Badri and Good, 1994; Gerritsen et al., 1994). We have previously shown that such a deficiency can be overcome if donor bones are engrafted in conjunction with BMT, since donor BM stromal cells migrate into the thymus, where they are engaged in positive selection. T cells in such mice can acquire donor-type major histocompatibility complex (MHC) restriction and can interact with both donor-type antigen-presenting cells (APCs) and B cells (Li et al., 2000). In these experiments, adult (8–10 weeks old) mice were used as recipients, and bones obtained from adult (8–10 weeks old) donor mice were engrafted into the mice that had received 2×10^6 T cell-, macrophage- and stromal cell-depleted BMCs by the conventional intravenous (IV) route.

In several earlier experiments, we have demonstrated that the co-administration of adult donor bones facilitates the acceptance of donor BMCs even in chimeric-resistant combinations, such as [normal → MRL/lpr] (Ishida et al., 1994) and [DBA/2 → B6] (Hisha et al., 1995). Donor-type stromal cells were detected in the BM adherent cells obtained from the treated chimeric mice. This indicates that BM stromal cells contained in the engrafted bones migrate into the recipient BM cavity and provide a suitable environment for donor hemopoietic stem cells (HSCs). We have also found that an MHC restriction exists between HSCs and BM stromal cells (Hashimoto et al., 1997; Sugiura et al., 2001); we grafted bones obtained from various mouse strains to one recipient mouse and BMCs were then injected by the IV route (Hashimoto et al., 1997). Significant cell accumulation of the injected BMCs was observed in the engrafted bones having the same MHC phenotype as the BMCs, whereas only a few BMCs were detected in the engrafted bones having different MHC phenotypes from the BMCs (Hashimoto et al., 1997). In accordance with this concept, clinical approaches using co-administration of donor bone fragments have been performed in patients who received non-myeloablative BMT and the facilitating effect of the grafted donor BM stromal cells was observed (Cahill et al., 2004; Jones et al., 2004).

More recently, we have found that the direct injection of donor BMCs (intra-bone marrow injection: IBM) produces more rapid reconstitution (including T-cell functions) and higher survival rates than IV injection even in chimerism-resistant combinations [normal → MRL/lpr mice] (Kushida et al., 2001). Moreover, we have shown that senile osteoporosis in SAMP6 mice can be prevented and treated effectively by IBM–BMT using normal mouse BMCs (Ichioka et al., 2002; Takada et al., 2006). In the recipient mice, the proliferation of donor stromal cells was also observed at the site of injection (Kushida et al., 2001; Takada et al., 2006). These results indicate the possibility that the stromal cells contained in donor BMCs play an important role in IBM–BMT. Therefore, we next performed simultaneous injection of donor BMCs and a stromal cell line (PA6 cells) into recipient bone cavity in a normal mouse combination. This approach allowed for a higher reconstitution of donor-type cells in the mice that had received IBM–BMT + injection of PA6 cells than in those that had received IV-BMT + injection of PA6 cells or that had received IBM–BMT alone (Zhang et al., 2004). The IBM–BMT group showed the highest survival rate of the three groups up to 60 days after BMT. When allogeneic BM adherent cells were used instead of the PA6 cells, a similar facilitating effect was observed in the IBM–BMT group (Zhang et al., 2004). These results indicate that co-administration of stromal cells in IBM–BMT provides a great advantage for the acceptance of donor cells.

Based on these findings, we hypothesized that complete reconstitution would be achieved in adult (more than 15 weeks old) mice if BMCs were administered by the IBM route in conjunction with bone grafts from younger mice. Therefore, in the present study, we first compared the *in vitro* proliferation and hemopoiesis-supporting ability of BM stromal cells obtained from suckling (2–3 days old) and young adult (4–5 weeks old) mice. Then we examined whether bone grafts could induce a complete reconstitution, including T-cell functions, in adult (more than 15 weeks old) mice that had received donor BMCs by the IV or IBM routes. To investigate precisely the facilitating effect of bone grafts, a lower number (5×10^6 per mouse) of whole BMCs was injected into the recipient mice, because we have administered 3×10^7 whole BMCs into the recipient mice (without donor bone grafts) in most of our previous experiments (Kushida et al., 2001; Ichioka et al., 2002; Takada et al., 2006). Moreover, we examined whether donor BM stromal cells migrate from the grafted bones into recipient BM, spleen and thymus after IBM–BMT and participate in positive selection.

Materials and methods

Animals

C57BL/6 (B6) (H-2^b), C3H/HeN (C3H) (H-2^k), and BALB/c (B/c) (H-2^d) mice were purchased from Clea Japan (Osaka, Japan) or Shizuoka Experimental Animal Laboratory (Shizuoka, Japan) and maintained in pathogen-free conditions in our animal facility. All the