A Review on “Modification of intra-bone marrow bone marrow transplantation: aiming for better bone marrow transplantation methods”

Abstract

We have developed a powerful bone marrow transplantation method, intra-bone marrow-bone marrow transplantation (IBM-BMT), in which donor BM cells (BMCs) are directly injected into the recipient’s BM, resulting in a rapid recovery of donor hematopoiesis and a reduction in the severity of GVHD. We subsequently developed more effective BMT protocols by modifying IBM-BMT. One method involves the simultaneous injection of BMCs and bone marrow stromal cells into the recipient’s bone marrow cavity. Another involves the retention of the injected BMCs in the recipient’s bone marrow cavity using collagen gel or magnetic beads plus a magnet. The last method is IBM-BMT using an inhibitor of apoptosis. These methods accelerate the hematopoiesis of donor bone marrow cells.

Keywords: intra-bone marrow-bone marrow transplantation (IBM-BMT), bone marrow stromal cell, collagen gel, magnetic beads, inhibitor for apoptosis, ZVAD-fmk.

1. INTRODUCTION:

In 1968, R.A. Good et al. successfully performed the first bone marrow transplantation (BMT) in humans [1]. Originally, BMT was developed in order to treat immunological and hematopoietic diseases, congenital immunodeficiency, leukemia, aplastic anemia, and so on [2-4]. And then, BMT has been used to solid tumors, autoimmune diseases, metabolic disorders, and so on [5-7]. Although procedures and management for BMT have been improved, the risks are still high [8]. Therefore, with a better safety profile and easy of application, BMT could be applied to a far wider range of diseases even in humans.

Upon BMT, donor bone marrow cells (BMCs) are injected into the recipient’s vein. However, we have developed a new BMT protocol, in which donor BMCs are injected directly into the recipient’s bone marrow cavity. We named this new BMT method intra-bone marrow bone marrow transplantation (IBM-BMT) [9]. IBM-BMT can reduce the dose of irradiation required as the pretreatment for BMT, induce rapid recovery of donor hematopoiesis, and suppress graft versus host disease (GVHD), in contrast to conventional bone marrow transplantation, in which donor BMCs are...
injected into the recipient’s peripheral vein [9]. We have subsequently attempted to further improve the IBM-BMT protocol.

2. IBM-BMT using donor BMCs and bone marrow stromal cells

We previously showed that MHC-matched bone marrow stromal cells can accelerate hematopoiesis of hematopoietic stem cells more efficiently than MHC-mismatched bone marrow stromal cells, both *in vivo* and *in vitro* [10, 11]. Based on this we transplanted BMCs from C57BL/6 mice (B6 mice) and cultured bone marrow stromal cells (BMSCs) of B6 mice into the bone marrow cavity of irradiated BALB/c mice [12]. The method showed better hematopoiesis of donor BMCs than the simple injection of only donor BMCs (Fig.1). These results suggest that the bone marrow stromal cells of the donor are able to facilitate the hematopoiesis of the donor BMCs in allogeneic BMT using the IBM-BMT method.

3. IBM-BMT using collagen gel

Since an abundance of blood vessels in the bone marrow, it was supposed that some of the injected BMCs would leak from the bone marrow into the peripheral blood during the IBM-BMT. Based on this hypothesis, it was suggested that the more donor BMCs could be retained in the recipient’s bone marrow cavity, the more effective would be the outcome of the IBM-BMT. We thus modified the protocol to ensure that more injected BMCs were retained in the recipient’s bone marrow cavity. One such modification involved the use of collagen gel, while another involved the use of magnetic beads and a magnet [13, 14].

We first discuss IBM-BMT using collagen gel[13]. The collagen gel we used is liquid at 4°C and a gel at 37°C. We started by suspending the donor BMCs in the collagen gel at 4°C and then warming the gel to 37°C. The warmed gel containing the donor BMCs was then injected into the bone marrow cavity of the irradiated recipient. Approximately twice the number of donor BMCs was still present in the injected recipient’s bone marrow cavity 1 hour after the IBM-BMT (Fig. 2). The recovery of donor hematopoiesis was more rapid with the IMB-BMT+collagen gel protocol than either intra-venous BMT (IV-BMT; conventional BMT) or IBM-BMT without the gel. Moreover, donor hematopoiesis expanded throughout the body more rapidly than IBM-BMT without the gel.

4. IBM-BMT using magnetic beads and a magnet

We also used magnetic beads and a magnet to help retain the injected donor BMCs in the bone marrow cavity of the recipient upon IBM-BMT [14]. To prepare magnetic bead-coated donor BMCs, donor BMCs were conjugated with biotin-labeled anti-CD45 antibody (Ab) and biotin-coated anti-Sca-1 Ab (Fig.3). Next, the BMCs were incubated with avidin-coated magnet beads. The thus-prepared magnetic bead-coated BMCs were then injected into the recipient’s bone marrow cavity. When the IBM-BMT was carried out, a magnet was positioned around the bone. Using this method, approximately twice the number of injected donor BMCs were still present in the injected recipient’s bone marrow cavity 1 hour after the IBM-BMT, and more CFU-S counts were observed using this protocol than when using only IBM-BMT. This method also allowed a reduction in the irradiation dosage of recipients as pretreatment for IBM-BMT.

Thus, both IBM-BMT using collagen gel and IBM-BMT using magnetic beads plus the magnet retained more donor BMCs than IBM-BMT alone, resulting in an acceleration of hematopoiesis from donor BMCs. These results confirmed our hypothesis that the more BMCs were retained in the bone marrow, the more rapidly hematopoiesis occurred.

It has been suspected that some donor BMCs become apoptotic during BMT. Therefore, to inhibit such apoptosis we injected an anti-apoptotic reagent, ZVAD.fmkk, into the donor mice before the preparation of the BMCs, or into the recipient mice after the IBM-BMT. Both methods accelerated the hematopoiesis of the donor BMCs: more CFU-S and more donor hematopoietic cells were found 12 days after IBM-BMT in the recipient mice in both methods than when using only IBM-BMT. This phenomenon was observed even one month after IBM-BMT, suggesting that the tendency observed 12 days after IBM-BMT would be observable over a longer term.
5. CONCLUSION:
As discussed, we have developed a number of modifications of the IBM-BMT protocol, these modifications being more effective in accelerating donor hematopoiesis than IBM-BMT alone. We would like to see the development of more effective and safer methods of BMT, based on our theory, than those currently carried out for humans.

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Conflict of Interest: The authors declare that the authors have no conflicts of interest.

7. REFERENCES:

Figure legends:
Fig.1 Cultured donor BMSCs accelerate hematopoiesis of donor hematopoietic cells (from Ref. 12). IBM-BMT was carried out from B6 mice to lethally irradiated C3H mice. Several kinds of control groups (intra-bone marrow injection of only saline, only latex beads, BMCs plus parafomaldehyde-
fixed BMSCs) were prepared. Twelve days after BMT, mice were euthanized and their colony forming units of spleen (CFU-S) were counted. Significantly more CFU-S counts were observed in mice injected with BMCs + BMSCs than those in mice injected with BMCs alone.

Fig. 2 Collagen gel efficiently helps retain donor cells in recipient BM (from Ref. 13).

C57BL/6 mice (B6 mice) were irradiated at 10 Gy one day before IBM-BMT. BMCs were obtained from eGFP tg-B6 mice (donor mice). The BMCs (1x10^7/10µl) suspended in PBS (conventional IBM-BMT) or collagen gel (IBM-BMT with collagen gel) were injected into the BM of the B6 mice. Control mice were injected with only PBS into the bone marrow. One hour after IBM-BMT, the mice were sacrificed and % of CD45^+ donor cells (A) and % of CD45^+lineage Sca-1^+ donor cells (B) in the bone marrow injected with donor bone marrow cells (BMCs) were analyzed by FACScan. n.=3 (control; mice injected with only PBS), 6 (conventional IBM-BMT) and 6 (IBM-BMT with collagen gel). The cells from control mice show autofluorescence. *p <0.05.
Fig. 3 Method for IBM-BMT using the combination of magnetic beads and magnet (from Ref. 14).

Hematopoietic nuclear cells of the BM express CD45 and HSCs express both CD45 and Sca-1 (A). First, donor BMCs were incubated with biotin-labeled anti-CD45 antibody (Ab) and biotin-labeled anti-Sca-1 Ab. Anti-CD45 Ab attached to nuclear cells of BMCs and anti-Sca-1 Ab attached to HSCs (B). Next, the cells were incubated with streptavidin-coupled magnetic beads (Dynabeads®), so that the magnetic beads became attached to the BMCs (C). In this figure, we show hematopoietic stem cells expressing Sca-1. The magnetic beads can attach to the hematopoietic stem cells through Sca-1 and CD45. The magnetic beads can also attach to other types of BMCs not expressing Sca-1 through CD45. The BMCs conjugated with magnetic beads were injected into the BM of the tibia and retained with the magnet (D, E). After IBM-BMT, the magnet was fixed to the tibia (F).
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