

# New Strategies for BMT and Organ Transplantation

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## Abstract

Bone marrow transplantation (BMT) is now one of the most powerful strategies for the treatment of hematologic disorders (leukemia, aplastic anemia, etc), congenital immunodeficiencies, metabolic disorders, and also autoimmune diseases. Using various autoimmune-prone mice, we have previously shown that conventional allogeneic (allo) BMT can be used to treat a range of autoimmune diseases. We have very recently established new strategies for BMT and organ grafts. For BMT, to minimize the contamination of BMCs with T cells from the peripheral blood, we developed, using cynomolgus monkeys, a “Perfusion Method” to replace the conventional aspiration method for collecting bone marrow cells (BMCs). We injected the BMCs collected this way directly into the bone marrow cavity of recipients that had received fractionated irradiation (intra-bone marrow [IBM] injection). This “IBM-BMT” was found to be effective in treating autoimmune diseases in radiation-sensitive and chimeric-resistant MRL/lpr mice. In addition, this strategy was found to be applicable for the transplantation of organs, such as the skin and pancreas islets in mice and rats. We believe that these strategies for BMT and organ transplantation herald a new era in transplantation.

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## 1. Introduction

We have previously shown using various autoimmune-prone mice that conventional allo BMT can be used to prevent and treat a range of autoimmune diseases [1-3]. These findings have recently been confirmed even in humans [4-6]. However, in humans, the success rate of BMT across major histocompatibility complex (MHC) barriers is lowered by graft-versus-host disease (GvHD), graft rejection, and incomplete T-cell recovery. Therefore, autologous BMT (auto BMT) or auto peripheral blood stem cell transplantation (PBSCT) is the preferred treatment for autoimmune diseases. There have, however, been reports on the rapid recurrence or persistence of autoimmune diseases after auto BMT or auto PBSCT [7]. Therefore, it is important to establish a safe new method for allo BMT.

Recently, we have found that in the injection of allogeneic bone marrow cells into the intra-bone marrow can induce donor-specific tolerance [8]. In this paper, we discuss the strategies we have developed for allo BMT and organ allografts.

## 2. New Strategies for Allo BMT

We have found that the MRL/lpr mouse, an animal model for autoimmune diseases, is a suitable model for establishing a safe new strategy for allo BMT, since the MRL/lpr mouse itself is radio-sensitive (8.5 Gy), while the abnormal hemopoietic stem cells of the MRL/lpr mouse are radio-resistant (>8.5 Gy); conventional BMT (8.5 Gy plus allo BMT) has a transient effect on autoimmune diseases, which recur 3 months after the BMT [9].

To prevent the recurrence of autoimmune diseases in MRL/lpr mice, we carried out BMT plus bone grafts to replace not only the hemopoietic cells but also the stromal cells with donor cells. This is because there is a MHC restriction between pluripotent hemopoietic stem cells (P-HSCs) and stromal cells [10,11]. MRL/lpr mice that had been irradiated (8.5Gy) and then reconstituted with C57BL/6 BMCs plus bone grafts survived more than 1 year [12,13].

Although we found that allogeneic BMT plus bone grafts (to recruit donor stromal cells) could prevent the development of autoimmune diseases in MRL/lpr mice,

this strategy (8.5 Gy/bone/BMT) was found not to be beneficial for the treatment of florid autoimmune diseases in MRL/lpr mice. MRL/lpr mice with small amounts of proteinuria (<+) can endure 8.5 Gy irradiation, whereas MRL/lpr mice with greater degrees of proteinuria ( $\geq$ ++) are more radiosensitive and are unable to withstand 8.5 Gy irradiation due to uremic enterocolitis. We therefore devised a new method, fractionated radiation (5 Gy $\times$ 2), which reduces acute radiation injury and therefore reduce the side effects of radiation and prevents graft rejection.

Recently, we have found that most donor HSCs are trapped and retained in the liver when they are injected either portal venously (PV) or even intravenously (IV) [14], and that the HSCs induce anergy to host CD8<sup>+</sup> T cells [15]. In addition, we found that a certain strategy (PV [on day 0] plus IV [on day 5] injections of donor whole BMCs) can induce persistent tolerance in the skin allograft system [16]. On the basis of these findings, we attempted to establish a new strategy for allogeneic BMT applicable to humans: fractionated irradiation (5.5 Gy $\times$ 2) and the PV administration of  $3 \times 10^7$  whole B6 BMCs. MRL/lpr mice (4-5 months of age) that had developed the symptoms of autoimmune diseases such as massive lymphadenopathy and proteinuria (more than 2.5+) were first treated with (5.5 Gy $\times$ 2+PV). As shown in Figure 1, more than 70% of the mice thus treated survived more than 1 year, indicating that this treatment has some effect on the treatment of autoimmune diseases. We next treated autoimmune diseases in MRL/lpr mice with [5.5 Gy $\times$ 2+PV+IV]. Thus-treated MRL/lpr mice showed a 100% survival rate 1 year after the treatment, indicating that the supplemental IV injection of BMCs is helpful for successful engraftment [17,18]. In contrast, all the recipients treated with (8.5 Gy+IV) died within 4 weeks because of the side effects of radiation, as previously described [19].

We analyzed the mechanism underlying the tolerance induced by the PV injection of BMCs and noted the importance of donor-derived stromal cells trapped in the liver, these cells facilitating the proliferation and differentiation of donor HSCs [20,21]. Based on these findings, we attempted to inject whole BMCs (including stromal cells) directly into the bone marrow cavity (intra-bone marrow [IBM] injection).

We reduced the radiation dose from 5.5 Gy $\times$ 2 to 5 Gy $\times$ 2 in order to compare the effect of IBM-BMT with that of PV-BMT. MRL/lpr mice treated with either (5 Gy $\times$ 2+PV) or (5 Gy $\times$ 2+PV+IV) showed survival rates of 30% and 50%, respectively (Figure 1). These findings indicate that the 5 Gy $\times$ 2 irradiation followed by the treatment with PV+IV is insufficient to prevent graft rejection. In contrast, all the recipients that had received "IBM-BMT" survived 48 weeks after the treatment without showing any signs of graft rejection or recurrence of autoimmune diseases even when treated with 5 Gy $\times$ 2+IBM (Figure 1). Furthermore, more than 85% of the MRL/lpr mice survived 30 weeks after the treatment, even when treated with 4.5 Gy $\times$ 2+IBM [8].

### 3. A New Strategy for Organ Allografts

We have previously shown that the combination of organ allografts and BMT from the same donors prevents rejection [22,23]. However, this method was not applicable to humans because of lethal levels of irradiation, and allogeneic BMT in humans has several other problems such as GvHD. As described here, we have recently found that "PV-BMT" is better than "IV-BMT" [20]. Accordingly, we attempted skin grafts using "PV-BMT".

Figure 2 shows that 100% skin allograft survival can be obtained by sublethal irradiation (7 Gy) followed by "PV-BMT" [24]. The recipient mice showed fully allogeneic chimerism, and spleen cells from the recipients showed tolerance to both donor-type and host-type MHC determinants in the assays for mixed lymphocyte reaction and generation of cytotoxic T-lymphocytes (CTLs). This method was found to be applicable to pancreas allografts [25]. This simple method should be applicable to human organ allografts. We next compared the effects of "IBM-BMT" on the induction of tolerance with the effects of "PV-BMT". "IBM-BMT" was found to be more effective in inducing tolerance than "PV-BMT", since the radiation dose could be reduced to 4.5 Gy $\times$ 2 (manuscript in preparation).

### 4. Prospects for "IBM-BMT"

In humans, the success rate of BMT across MHC barriers is lowered by GvHD, graft rejection and incomplete T cell recovery. To prevent GvHD, we attempted to minimize the contamination of BMCs with T cells from the peripheral blood when donor BMCs are collected, finally establishing a new "Perfusion Method" using cynomolgus monkeys. In this method, two BM puncture needles are inserted into a long bone such as the humerus, femur, or tibia. One needle is connected to an extension tube and the end of the tube is inserted into a culture flask to collect the BM fluid (Figure 3). The other needle is connected to a syringe containing 30 ml of phosphate-buffered saline. The solution is ejected gently from the syringe into the medullary cavity, and the medium containing the BM fluid is collected into the culture flask. There is significantly less contamination with the peripheral blood, as determined from the frequencies of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, when using this method (<6%) than when using the conventional method (>20%) consisting of multiple BM aspirations from the iliac crest. Furthermore, the number and progenitor activities of the cells harvested using our "Perfusion Method" are greater than those harvested using the conventional aspiration method [26].

Combining IBM-BMT with the "Perfusion Method" seems to be the best strategy for allogeneic BMT: (1) No GvHD develops even if T cells are not depleted from the bone marrow; (2) no graft failure occurs even if the dose of radiation as the conditioning for BMT is reduced to 5 Gy $\times$ 2; (3) hemopoietic recovery is rapid; and (4) the restoration of T-cell functions is complete

even in donor-recipient combinations across the MHC barriers. This IBM BMT is therefore applicable to humans, since intraosseous (io) infusion (IBM injection) is an established method for administering fluids, drugs, and blood to critically ill patients, particularly infants [27]. Indeed, Hagglund et al. recently compared the effectiveness of io infusion with that of iv infusion in human allogeneic BMT; they concluded that allogeneic BMT can be safely performed by io infusion, but the incidence of acute and chronic GvHD, transplantation-related mortality, and survival rates are similar [28]. However, they aspirated the donor BMCS from the iliac bones (but not long bones) and infused these BMCs into the iliac bones of the recipients.

We believe that this "Perfusion Method" would also contribute to gene therapy and organ allografts in conjunction with BMT even in humans. We are now determining in cynomolgus monkeys which conditioning is best for IBM-BMT or organ transplantation in conjunction with IBM-BMT.

We have very recently found using TsK mice [29] that IBM-BMT can be used to treat not only scleroderma but also emphysema in TsK mice [30]. In addition, we have found that IBM-BMT can be used to prevent and treat osteoporosis in SAMP6 mice [31]. We therefore believe that this method (IBM-BMT in conjunction with the Perfusion Method) will become a powerful new strategy for the treatment of various intractable diseases. Furthermore, we believe that this method would become a valuable strategy in regeneration therapy for injured organs and tissues (osteoporosis, emphysema, myocardial infarction, cerebral infarction, Alzheimer's disease, etc.), since IBM BMT can efficiently reconstitute the recipient with both donor-derived hemopoietic stem cells and mesenchymal stem cells.

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**Figure 1.** Treatment of autoimmune diseases in MRL/lpr mice by various strategies. IBM-BMT is more effective than PV-BMT or IV-BMT.

**Figure 2.** Successful skin allografts by various strategies. Successful skin allografts can be achieved by IBM-BMT; the radiation dose can be reduced to 4.5 Gy $\times$ 2 (sublethal dose).

**Figure 3.** The "Perfusion Method". Two needles are inserted into the humerus. One needle is connected to an extension tube and the end of the tube is inserted into a culture flask to collect the BM fluid. The other needle is connected to a syringe containing 30 ml of PBS. The solution is ejected from the syringe into the BM cavity. The medium containing BM fluid is collected into the flask.