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Bone marrow transplantation improves outcome in a mouse model of congenital muscular dystrophy

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Abstract **We examined whether pathogenesis in dystrophin-deficient (*mdx*) mice and laminin-α2-deficient (*dy*) mice is ameliorated by bone marrow transplantation (BMT). Green fluorescent protein (GFP) mice were used as donors. In *mdx* mice, BMT failed to produce any significant differences in muscle pathology, although some GFP-positive fibers with restored dystrophin expression were observed. In contrast, in the *dy* mice, BMT led to a significant increase in lifespan and an increase in growth rate, muscle strength, and respiratory function. We conclude that BMT improved outcome in *dy* mice but not *mdx* mice.**

Keywords: Bone marrow transplantation; Muscular dystrophy; *mdx* mouse; *dy* mouse; Laminin α2; Basal lamina

1. Introduction

The muscular dystrophies are groups of inherited myogenic disorders characterized by progressive muscle wasting and weakness of variable distribution and severity. Two major types of severe muscular dystrophy, Duchenne muscular dystrophy (DMD) and congenital muscular dystrophy, have been identified [1]. DMD is caused by mutations of the dystrophin gene [2]. Most cases of congenital muscular dystrophy are caused by mutations in the laminin-α2 chain (merosin) gene. This disease has been termed merosin-deficient congenital muscular dystrophy (MCMD) or MDC1A [3]. Pathogenesis of dystrophin-deficient or laminin-α2-deficient muscular dystrophy can be studied in mouse models [4]. Loss of dystrophin protein is observed in the *mdx* mouse, the mouse model of DMD [5]. The *dystrophia muscularis* (*dy*) mouse has spontaneous mutation in the *Lama2* gene encoding laminin-α2 and is used as a model of MDC1A [6,7]. Although some potential treatments including pharmacologic methods, gene therapy, and cell therapy have been tried, there are no effective therapeutic approaches for muscular dystrophy at present [8].

Bone marrow transplantation (BMT) is an established clinical procedure used to treat various human diseases. Adult bone marrow (BM) cells contain mesenchymal stem cell progenitors, which can give rise to osteocytes, chondrocytes, adipocytes, and myocytes [9,10]. BM is also a promising source of myogenic stem cells [11]. Recently, several investigators have reported that transplanted BM cells participate in the muscle regeneration process in irradiated recipient mice [12–15] or DMD patient [16]. However, analyses of these studies are often limited to histopathologic assessments.

In the present study, we examined the therapeutic effect of whole BMT on muscular dystrophy model mice by evaluating clinical phenotypes such as body weight, lifespan, muscle strength, and respiratory function as well as histopathology. We also compared the therapeutic effect of whole BMT on two distinct models of muscular dystrophy, *mdx* and *dy* mice. Our results showed that BMT improved outcome in *dy* mice but failed to affect pathology of *mdx* mice. Thus a therapeutic approach of transplanting BM cells could be considerable benefit in MDC1A.

2. Materials and methods

2.1. Mice

The C57BL/6 (wild-type) mice were purchased from Clea Japan (Tokyo, Japan). The *mdx* mice (of C57BL/10 background) were provided by Central Institute for Experimental Animals (Kanagawa, Japan). The laminin-α2-deficient *Lama2*−/− mice (*dy*) mice and the EGFP transgenic (GFP-Tg) mice with a C57BL/6 background [17] were purchased from Jackson Laboratory (Bar Harbor, Maine, USA). All experiments involving animals were performed under the guidelines of the Institutional Animal Care and Research Advisory Committee, Kawasaki Medical School.

2.2. Bone marrow reconstitution

BM chimeras were established by following the method of Fukada et al. [15] with modifications. Briefly, adult (8-week-old) wild-type, *mdx* or *dy* mice received 9 Gy TBI (X-ray), split into two doses separated by 3 h to minimize gastrointestinal toxicity. BMT was performed according to a standard protocol described previously [18,19]. Recipient mice were injected with 5×10^6 T cell-depleted BM cells. T cell depletion of donor BM cells was performed using anti-CD90-MicroBeads and an AutoMACS system (Miltenyi Biotec, Auburn, CA, USA) according to the manufacturer's instructions. No unfavorable results such as GVHD were observed in recipient mice received BM cells from GFP mice.

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Abbreviations: BMT, bone marrow transplantation; DMD, Duchenne muscular dystrophy; MDC1A, congenital muscular dystrophy type 1A; SpO_2, arterial hemoglobin saturation

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2.3. Histology and immunohistochemistry

Cryosections of diaphragm muscle were prepared as described previously [20]. For immunohistochemical analysis, sections were immunostained with a rabbit polyclonal antibody against GFP (MBL, Nagoya, Japan) or a monoclonal antibody against C-terminus of dystrophin (NCL-DYS2; Novocastra, Newcastle, United Kingdom), laminin-α2 (merosin) (clone 4H8-2; Sigma–Aldrich, St. Louis, MO, USA) followed by fluorescein isothiocyanate-conjugated secondary antibodies according to the MOM procedure (Vector Laboratories, Burlingame, CA, USA). The slides were mounted with VECTASHIELD plus DAPI (Vector Laboratories). The fluorescence images were recorded photographically using a microscope (Nikon, Tokyo, Japan) and analyzed with Lumina Vision software (Mitani Corporation, Fukui, Japan).

2.4. Grip strength test and pulse oximetry

Peak grip strength (g) was measured using an MK-380S automated grip strength meter (Muromachi Kikai, Tokyo, Japan) as described previously [21]. Arterial hemoglobin saturation (SpO_2) was measured with Masimo SET (Masimo Corp., Irvine, CA, USA) [22].

2.5. Statistics

Statistical analysis was performed on paired observations using Bonferoni's test after one-way ANOVA.

3. Results

3.1. BMT promotes survival and growth of laminin-α2-deficient mice

We first examined whether BMT affects lifespan in the *mdx* mice and *dy* mice. Kaplan–Meier survival curves revealed that a large percentage of control *dy* mice died around the first 20 weeks after birth (Fig. 1), whereas *dy* mice that received BMT survived up to 40 weeks, or almost double the lifespan of control *dy* mice. In *mdx* mice, we kept both groups of mice up to 2 years and found no difference for lifespan between BMT and control groups. These results indicate that BMT eliminated early death of *dy* mice.

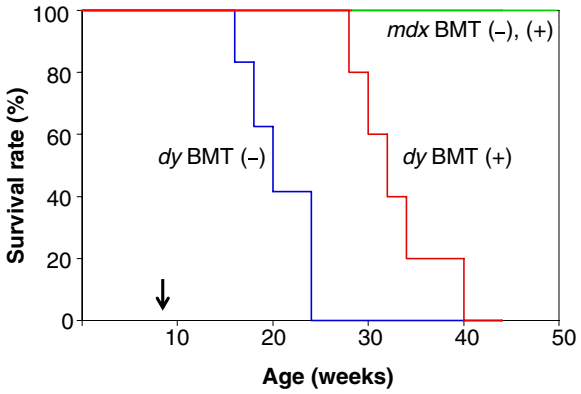

Fig. 1. Kaplan–Meier survival curves for BMT or non-treated control groups of each model of mice ($n = 10$, each). Arrow indicates 8 weeks of age at the time of BMT was performed. BMT (−), control group; BMT (+), treated group.

We further examined progressive changes in body weight of these models. BMT did not affect the growth rate of *mdx* mice in both males and females (Fig. 2A). The *dy* mice that received BMT lost weight right after BMT but gained weight more quickly and grew significantly larger than non-treated littermates (Fig. 2B). This tendency was found in both males and females. Thus, in addition to increasing lifespan, BMT improved the growth of *dy* mice.

3.2. BMT dose not significantly alter pathology in mdx muscle

We then questioned weather BMT improves muscle pathology of *mdx* mice. We examined diaphragm muscle, known to

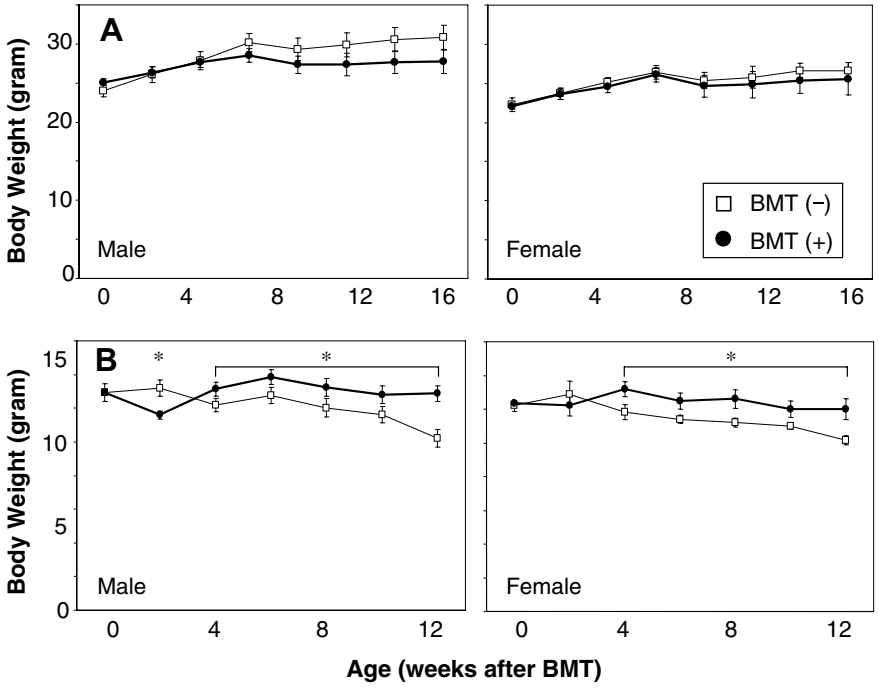

Fig. 2. (A) Growth curves for *mdx* mice between the control and BMT groups in males or females up to 16 weeks after BMT ($n = 5$, each). (B) Growth curves for *dy* mice until 12 weeks after BMT ($n = 5$, each). Data are expressed as means ± S.D. $^{*}P < 0.05$, Bonferoni's test after one-way ANOVA. BMT (−), control group; BMT (+), treated group.