Reduction of lysosomal storage in murine mucopolysaccharidosis type VII by transplantation of normal and genetically modified macrophages

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This study examined the ability of macrophages to serve as target cells of gene therapy for mucopolysaccharidosis (MPS) type VII using a murine model. Bone marrow cells were harvested from syngeneic normal mice and differentiated to macrophages. These cells were given to nonmyeloablated MPS VII mice. After transplantation, donor cells populated the liver and spleen. The pathologic improvement at day 38 after transplantation was significant and glycosaminoglycan storage was reduced. To develop gene therapy using this system, a retroviral vector expressing human β-glucuronidase (HBG) was used to infect macrophages cultivated from MPS VII mice and given to nonmyeloablated MPS VII mice. At 38 days after transplantation, HBG-positive cells were still observed histochemically and pathologic improvement was significant. These observations suggest that macrophage transplantation is a promising method for treatment of murine MPS VII without myeloablation, and macrophages may be good target cells for ex vivo gene therapy for MPS VII. (Blood. 2000;95:3631-3633)

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Introduction

Mucopolysaccharidosis type VII (MPS VII), which is also known as Sly syndrome, is a lysosomal storage disease caused by deficiency of human β-glucuronidase (HBG). This results in accumulation of glycosaminoglycans (GAGs) in various tissues.[1] Murine models of MPS VII are available, which exhibit biochemical and clinical phenotypes similar to those of the human disease.[2,3] Using this model, various gene therapy approaches, including gene transfer to hematopoietic stem cells (HSC), have been reported.[4] So far, gene transfer to HSC appears the most practical gene therapy approach to treat lysosomal storage disease, including MPS VII. However, gene transfer to human HSC by various gene transfer methods has been inefficient. In a clinical trial of gene therapy for a lysosomal storage disease based on transferring the therapeutic gene to human HSC, the transduction efficiency was too low to alter the disease phenotype.[5] To overcome the problems of gene transfer to HSC, we studied the usefulness of macrophages as target cells for MPS VII gene therapy.

Materials and methods

Mice

Breeding pairs of (+/−) mice (B6.C-H2^{bml}/BY Bir-gus^{mps}/+) were purchased from the Jackson Laboratory (Bar Harbor, ME) and bred. Homozygous mutants (−/−), heterozygous (+/−), and wild-type (+/+) animals were identified by DNA analysis.[6,7]

Macrophage culture

Macrophages from bone marrow cells were cultivated from normal mice (+/+) and MPS VII mice (−/−) as described.[8,9] Briefly, bone marrow cells were harvested from femoral bones of mice and seeded onto unprocessed 100-mm polystyrene dishes (2×10^5 cells/dish) in 50% Dulbecco's modified Eagle's medium, 10% fetal calf serum, 20% horse serum, and 20% L929 conditioned medium (macrophage medium). Two weeks after the initiation of culture, the adherent cells were collected and suspended in phosphate-buffered saline. We usually obtained 3.0×10^6 adherent cells from one 100-mm dish (originally seeded at 2×10^5 bone marrow cells). More than 95% of the adherent cells were positive for macrophage markers (CD18, CD11b, F4/80 antigen) (data not shown).

Transduction of macrophages by retroviral vector (MFG-HBG)

One day before harvesting of bone marrow cells, the medium from retroviral producer cells was changed to macrophage medium. The retroviral vector has been described in detail previously.[10] The next day, the bone marrow cells of MPS VII mice (−/−) were harvested and 2×10^5 bone marrow cells were suspended in 7 mL of filtered (0.45 μm pore size) macrophage medium conditioned by retroviral vector-producer cells containing 8 μg/mL of polybrene and plated in 100-mm unprocessed dishes. The next day, 4 mL of medium containing nonadherent cells was removed and mixed with 4 mL of filtered macrophage medium conditioned by retroviral vector-producer cells. Polybrene was added at final concentration of 8 μg/mL. The mixture (total 8 mL) was centrifuged at 2400*g* for 2 hours at 4°C. After centrifugation, 4 mL of medium was discarded without disturbing the cell pellet; then the cells were suspended and added back to dishes. This method was repeated for 5 consecutive days, then the culture was continued for 2 weeks. Just before transplantation into mice, HBG gene expression was determined by HBG activity assay and reverse transcriptase-polymerase chain reaction (RT-PCR).

Transplantation to MPS VII mice

Aliquots of 3.6×10^6 normal macrophages or genetically modified macrophages were infused intravenously into 8- to 10-week-old nonmyeloablated

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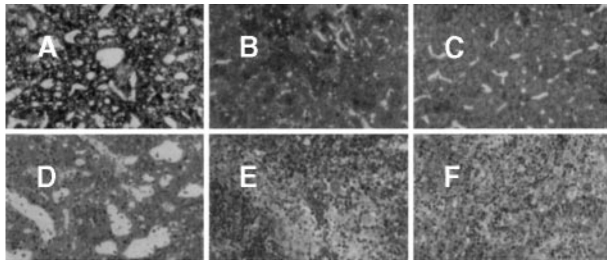

Figure 1. Live and spleen at day 38 after transplantation. The liver of untreated, age-matched MPS VII mice (A) showed lysosomal distention in Kupffer cells and a small amount of storage in hepatocytes. At day 38 after transplantation of normal macrophages (B), Kupffer cell storage was markedly reduced. However, reduction of storage in hepatocytes was less marked. The untreated MPS VII mouse spleen showed abundant lysosomal storage in sinus-lining cells in the red pulp (D). At day 38 after transplantation of normal macrophages, there was a similar marked reduction in the amount of lysosomal storage (E). Thirty-eight days after transplantation of genetically modified macrophages, histologic findings in the liver and spleen were similar to those of mice that received normal macrophages (C and F) (A-F, toluidine blue; original magnification × 200).

MPS VII mice. The results of our preliminary studies indicated that the mice tolerated this number of cells well. Tissues were isolated for analysis at 7 or 38 days after transplantation.

Histologic and biochemical studies

The activity of HBG in the liver and spleen was also assayed histochemically.[7] Thin sections (0.5 µm) of tissue were stained with toluidine blue to evaluate lysosomal storage.[7] HBG activity in the tissues was assayed as described.[11] The concentration of GAGs in the liver and spleen was measured as described.[12]

Results and discussion

Human HSC are resistant to retoroviral infection.[5] To seek an alternative approach for the treatment of MPS VII, we used macrophages as target cells for gene transfer instead of HSC.

Recently, Kennedy et al[13] demonstrated that murine macrophages cultured in vitro can enter tissues and engraft after transplantation. Moreover, Hahn et al[14] demonstrated that expression of the therapeutic gene in macrophage lineage cells was therapeutic in a mouse galactosialidosis model. These observations supported our strategy for treatment of MPS VII by transplantation of normal or genetically modified macrophages. At 7 days after injection of normal macrophages into nonmyeloablated MPS VII mice, the HBG activities in the liver and spleen were increased (Table 1) from 0.84 ± 0.75 to 32.9 ± 8.6 nmol/h/mg and from 0.44 ± 0.39 to 35.0 ± 22.3 nmol/h/mg, respectively. Histochemical analysis of HBG activity after transplantation indicated that many enzyme-competent macrophages entered the liver and spleen (data not shown). In contrast to the liver and spleen, increases in enzymatic activities in other tissues such as the brain, lung, kidney, and heart were minimal (data not shown). Although the HBG activity in the liver and spleen subsequently fell to 3.6 ± 1.5 and 2.3 ± 0.6 nmol/h/mg, respectively, by 38 days, a number of HBG-positive cells were still observed histochemically and pathologic improvement was significant. Light micrographs of the liver and spleen at day 38 are shown in Figure 1. The abundant lysosomal storage in Kupffer cells was reduced in treated animals, with small amounts of storage still seen in hepatocytes (Figure 1B). In the spleen, the abundant lysosomal storage in red pulp was also reduced (Figure 1E).

We analyzed the amounts of various GAGs in the liver and spleen to confirm the histologic data. Although levels of most of the GAGs analyzed were reduced in both the liver and spleen, they were still above the normal range (Table). These findings were consistent with those of histochemical analysis. Recently, another laboratory independently came to a conclusion similar to ours.[15]

We extended this study by using transplanted macrophages as a vehicle for gene therapy of murine MPS VII. We infected macrophages cultivated from MPS VII homozygous mutant mice (−/−) with an MFG-HBG retroviral vector, and transplanted these cells into nonmyeloablated MPS VII mice. The HBG activity in transduced macrophages cultivated from MPS VII mice was increased from 92 ± 62 (n = 4) to 10 265 ± 2325 (n = 4) nmol/h/mg, and was higher than that in macrophages cultivated from normal mice (+/+) (8066 ± 1537 nmol/h/mg, n = 5). The human HBG-specific transcript was detected by RT-PCR using human HBG-specific primers (data not shown). HBG activities in the liver and spleen from MPS VII mice that received genetically modified macrophages were increased at day 7 after transplantation (28.5 ± 4.3 nmol/h/mg and 32.4 ± 7.3 nmol/h/mg, respectively). These values were almost the same as those observed in animals that received normal macrophages. The activities subsequently fell by 38 days (3.9 ± 0.8 nmol/h/mg in the liver and 2.3 ± 0.8 nmol/h/mg in the spleen). However, HBG was detectable histochemically at 38 days after transplantation (data not shown) and pathologic improvement was significant (Figure 1C and F). The extensive lysosomal storage in the liver and spleen was reduced. Levels of GAGs in both the liver and spleen were reduced in mice receiving transduced macrophages, but were still above the normal range (Table 1).

Our observations indicate that macrophages could be alternative target cells for gene therapy of MPS VII and other storage disorders. An important advantage of this approach is that this

Table. HBG activity in organs after treatment and effect of treatment on GAG levels in liver and spleen

Genotype	Transplanted macrophages	Days after infusion	HBG activity in organs		GAG level in liver			GAG level in spleen		
			Liver	Spleen	CS	DS	HA	CS	DS	HA
+/−(n = 5)	—	—	1030 ± 73	1369 ± 459	0.05 ± 0.01	0.04 ± 0.01	0.01 ± 0.00	0.68 ± 0.19	0.14 ± 0.06	0.06 ± 0.02
−/−(n = 5)	—	—	0.84 ± 0.75	0.44 ± 0.39	1.92 ± 0.78	0.39 ± 0.07	2.00 ± 0.29	5.78 ± 2.70	1.36 ± 0.53	2.21 ± 1.00
−/−(n = 4)	Normal	7	32.9 ± 8.6	35.0 ± 22.3	nd	nd	nd	nd	nd	nd
−/−(n = 3)	Normal	38	3.6 ± 1.5	2.3 ± 0.6	0.37 ± 0.09*	0.07 ± 0.01*	0.14 ± 0.06*	3.64 ± 1.04	0.54 ± 0.13*	0.28 ± 0.13
−/−(n = 3)	Transduced	7	28.5 ± 4.3	32.4 ± 7.3	nd	nd	nd	nd	nd	nd
−/−(n = 3)	Transduced	38	3.9 ± 0.8	2.3 ± 0.8	0.41 ± 0.10	0.14 ± 0.03*	0.20 ± 0.07*	2.80 ± 0.50	0.53 ± 0.12*	0.24 ± 0.09*

HBG activity is expressed as nmol/h/mg. GAGs levels in liver and spleen are expressed in µg/mg of wet tissue. The data presented are mean ± SD.
CS, chondroitin sulfate; DS, dermatan sulfate; HA, hyaluronic acid; nd, not determined.
*Indicates significant reduction ($P < .05$, 2-sample *t* test) of GAGs in tissues.

procedure can be carried out without myeloablation. The main drawbacks of this approach were that terminally differentiated macrophages have a limited life span, and transplanted macrophages did not migrate to the brain. We are currently investigating approaches to overcome these limitations of our strategy.

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