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Successful modulation of type 2 diabetes in db/db mice with intra-bone marrow–bone marrow transplantation plus concurrent thymic transplantation

Ming Li^a, Nader G. Abraham^b, Luca Vanella^b, Yuming Zhang^a, Muneo Inaba^c, Naoki Hosaka^c, Sho-Ichi Hoshino^d, Ming Shi^a, Yoko Miyamoto Ambrosini^e, M. Eric Gershwin^e, and Susumu Ikehara^{a,*}

^aDepartment of Stem Cell Disorders, Kansai Medical University, Moriguchi City, Osaka 570-8506, Japan

^bDepartment of Physiology and Pharmacology, University of Toledo, Toledo, OH, USA

^cFirst Department of Pathology, Kansai Medical University, Moriguchi City, Osaka, Japan

^dThird Department of Internal Medicine, Kansai Medical University, Moriguchi City, Osaka, Japan

^eDivision of Rheumatology, Allergy and Clinical Immunology, University of California at Davis School of Medicine, Davis, California USA

Abstract

There is increasing evidence that both autoimmune and autoinflammatory mechanisms are involved in the development of not only type 1 diabetes mellitus (T1 DM), but also type 2 diabetes mellitus (T2 DM). Our laboratory has focused on this concept, and in earlier efforts replaced the bone marrow cells (BMCs) of leptin receptor-deficient (db/db) mice, an animal model of T2DM, with those of normal C57BL/6 (B6) mice by IBM–BMT. However, the outcome was poor due to incomplete recovery of T cell function. Therefore, we hypothesized that intra-bone marrow–bone marrow transplantation plus thymus transplantation (IBM–BMT + TT) could be used to treat T2 DM by normalizing the T cell imbalance. Hence we addressed this issue by using such dual transplantation and demonstrate herein that seven weeks later, recipient db/db mice manifested improved body weight, reduced levels of blood glucose, and a reduction of plasma IL-6 and IL-1 β . More importantly, this treatment regimen showed normal CD4/CD8 ratios, and increased plasma adiponectin levels, insulin sensitivity, and the number of insulin-producing cells. Furthermore, the expression of pancreatic pAKT, pLKB1, pAMPK and HO-1 was increased in the mice treated with IBM–BMT + TT. Our data show that IBM–BMT + TT treatment normalizes T cell subsets, cytokine imbalance and insulin sensitivity in the db/db mouse, suggesting that IBM–BMT + TT is a viable therapeutic option in the treatment of T2 DM.

Keywords

Type 2 diabetes; db/db/ mice; Intra-bone marrow–bone marrow; transplantation; Thymic transplantation

*Corresponding author. Tel.: +816 6993 9625; fax: +816 6994 9627, ikehara@takii.kmu.ac.jp (S. Ikehara).

1. Introduction

There is a virtual epidemic of type 2 diabetes, and although the mechanisms for this increase are not entirely clear, it has become the focus of both genetic and environmental research [1]. Clearly, inflammation has a critical role in the development of metabolic diseases, including obesity and T2 DM [1]. Recently, it has been shown that obese adipose tissue activates CD8T cells, resulting in promoting the recruitment and activation of macrophages in the adipose tissue [2]; macrophages have been shown to infiltrate the adipose tissue in mice and humans [3]. Adipocytes regulate and mediate inflammatory cytokines such as tumor necrosis factor- α (TNF α), IL-6, matrixmetalloproteinases (MMPs), peroxisome proliferation activated receptor- α (PPAR- α) and fatty acid-binding protein 4. These cytokines inhibit or enhance each other, and their activities contribute to insulin resistance [4]. As such, both an autoinflammatory as well as an autoimmune response are involved in the pathogenesis of T2 DM.

Bone marrow transplantation (BMT) has been demonstrated to treat hematopoietic disorders, metabolic disorders and autoimmune diseases [5–19]. We have recently found that intra-bone marrow-BMT (IBM-BMT) treatment is an advantageous strategy for allogeneic BMT, compared with conventional intravenous BMT [23], since IBM-BMT can replace not only hemopoietic cells (including hemopoietic stem cells:HSCs) but also stromal cells (including mesenchymal stem cells:MSCs). In addition, we have very recently found that thymus transplantation combined with BMT (BMT + TT) is a powerful strategy to ameliorate thymic involution in recipient mice due to aging or irradiation [20–22].

Based on these findings, we carried out IBM-BMT in combination with newborn thymus transplantation (TT) in db/db mice. We here demonstrate that, after IBM-BMT + TT treatment in db/db mice, insulin sensitivity increases and blood glucose levels decrease, resulting from the normalization of balance of lymphocyte subsets and cytokines, followed by enhanced expression of pAKT, pLKB1, pAMPK, insulin receptor phosphorylation and HO-1. This suggests that the maintenance of the balance of lymphocyte subsets and cytokine production by IBM-BMT + TT treatment is essential for the amelioration of T2 DM in db/db mice.

2. Materials and methods

2.1. Animals

Five-week-old BKS.Cg-m+Lepr^{db}/+Lepr^{db}/J (H-2k^d) (db/db) mice, BKS. Cg-m+Lepr^{db}/J(H-2k^d) (lean) mice and C57BL/6 (B6) (H-2k^b) mice were purchased from Charles River Laboratories (Yokohama, Japan) and SLC (Shizuoka, Japan) and maintained in animal facilities under specific pathogen-free conditions. All procedures were performed under protocols approved by the Institutional Animal Care and Use Committee at Kansai Medical University. Body weight and blood glucose levels were measured each week. Six-week-old mice with blood glucose levels higher than 250 mg/dl on two consecutive measurements were considered to have the onset of diabetes, and these mice were separated into three groups ($n = 6$ in each group): non-treated, treated with IBM alone, and treated with IBM–

BMT + TT. All data were collected at 4 weeks and 7 weeks after treatment. The same experiment was repeated three times.

2.2. IBM–BMT + TT

The mice received fractionated irradiation twice a day ($5.0 \text{ Gy} \times 2$, 4-hour interval). One day after the irradiation, whole BMCs from B6 mice were injected into the recipient mice (1×10^7 /mouse) by IBM–BMT using our previously described method [23]. Simultaneously, the newborn thymus from B6 mice was grafted under the renal capsule of the left kidneys of the recipient mice.

2.3. Flow cytometric analyses

The peripheral blood mononuclear cells were obtained from the tail vein of the recipients 30 days after transplantation. These cells were stained with antibodies against PE-H-2k^b, FITC-CD4, FITC-CD8a, FITC-B220 and FITC-CD11b (BD Bioscience Pharmingen, San Diego, CA) for 30 min on ice. After washing twice with 2% FCS/PBS and lysing red blood cells, the 10000 events acquired were analyzed by FACScan (BD Bioscience Pharmingen). Isotype-matched immunoglobulins were used as controls.

2.4. Insulin tolerance test

Insulin tolerance was tested at 7 weeks after treatment. After a 6-h fast, mice were injected intraperitoneally with insulin (2.0 units/kg). Blood samples were taken at various time points (0–90 min) and blood glucose levels were measured.

2.5. Cytokine and insulin measurements

Adiponectin, IL-6, IL-1 β and TNF- α were determined in mouse plasma using an ELISA assay (R&D Systems, Inc. MN and Invitrogen Corporation CA). Insulin was measured using an ELISA kit (Morinaga, Yokohama, Japan).

2.6. Immunochemistry

The pancreata, adipose tissue, and livers of the recipients, lean and db mice were removed 2 months after the transplantation. After the tissues were fixed in 10% formalin for 24 h at room temperature, they were embedded in paraffin. The sections (3 mm thickness) were stained with hematoxylin and eosin. To confirm the presence of glycogen deposits, they were stained with Periodic Acid Schiff (PAS) after diastase digestion. The pancreata were stained with polyclonal guinea pig anti-swine insulin antibody (N1542, Dako Cytomation, CA). The stained sections were examined on a microscope. The size of adipocytes was randomly measured using DP2-BSW application software (Olympus, Japan).

2.7. Mitogen response

The spleen was removed from the db/db mice at 7 weeks after treatment. A total of 2×10^5 splenocytes collected from chimeric mice, and untreated B6 and db/db mice as responders, were plated in 96-well plates (Corning Glass Works, Corning, NY) containing 200 μl of RPMI 1640 medium (Nissui Seiyaku, Tokyo, Japan) including 2 μl glutamine and 10% FCS. Responder cells were incubated with 2.5 $\mu\text{g/ml}$ of Con A (Calbiochem, San Diego, CA) or

25 µg/ml of lipopolysaccharide (Difco Laboratories, Franklin Lakes, NJ) for 72 h. 20 µl of 0.5 µCi of ³[H]-TdR (New England Nuclear, Cambridge, MA) was introduced during the last 18 h. Incorporation of ³[H]-TdR was measured using Microbeta TriLux (Perkinelmer, Wellesley, MA).

2.8. Western blot analysis of pancreata pLKB1, HO-1, AMPK, pAMPK, AKT, pAKT and insulin receptor phosphorylation

At sacrifice, pancreata were dissected, pooled for each mouse and used to measure signaling molecules. Specimens were stored at -140 °C until assayed. Frozen pancreatic tissues were pulverized under liquid nitrogen and placed in a homogenization buffer (mmol/l: 10 phosphate buffer, 250 sucrose, 1 EDTA, 0.1 PMSF and 0.1% v/v tertigol, pH 7.5). Homogenates were centrifuged at 27,000×g for 10 min at 4 °C, supernatant was isolated and protein levels were visualized by immunoblotting with antibodies. Antibodies against pLKB1, AMPK, pAMPK, AKT, pAKT and HO-1 were obtained from Cell Signaling Technology, Inc. (Beverly, MA). Antibodies were prepared by dilution of HO-1, pAMPK, pAKT and insulin receptor as we described previously [24,25].

2.9. Statistical analysis

Statistical significance between experimental groups was determined by the Fisher method of analysis of multiple comparisons ($p < 0.05$ was regarded as significant). For comparison between treatment groups, the null hypothesis was tested by either a single-factor ANOVA for multiple groups or unpaired *t* test for two groups. Differences between experimental groups were evaluated with ANOVA with Bonferroni corrections. Statistical significance was regarded as significant at $p < 0.05$.

3. Results

3.1. Body weight, blood glucose levels, insulin sensitivity, and plasma adiponectin, insulin, IL-6 and IL-1β levels

In our preliminary experiments, we carried out IBM-BMT alone (without TT). The IBM-BMT-treated db/db mice showed decreased blood glucose levels (<150 mg/ml) one week after the treatment but rapid increases in blood glucose levels 2 weeks after the treatment; the mice became susceptible to severe infection due to a rebound phenomenon, and died. Therefore, in the present study, we carried out IBM-BMT + TT, and non-treated db/db mice were used as the control.

As seen in Fig. 1A, a gain in body weight was prevented in the db/db mice treated with IBM-BMT + TT at each time point after the treatment from 6 weeks to 13 weeks, in contrast to the age-matched non-treated db/db mice ($*p < 0.05$ at each time point). There was no significant difference between the two groups in food intake (5.4 ± 0.1 vs 5.6 ± 0.2 g per mouse), although food intake was only 3.3 ± 0.1 g per lean mouse. To our surprise, the fasting blood glucose levels (Fig. 1B) significantly decreased in the db/db mice treated with IBM-BMT + TT (7 weeks after the treatment), compared with non-treated db/db mice (165.3 ± 5.84 vs 522.7 ± 40.22 mg/dl, $p < 0.001$).

As shown in Fig. 1C, insulin administration to the db/db mice treated with IBM-BMT + TT produced a rapid decrease in the blood glucose levels, suggesting improved insulin sensitivity after the treatment. Blood glucose levels at all time points in the db/db mice treated with IBM-BMT + TT were lower than those in non-treated db/db mice ($p < 0.05$).

As shown in Fig. 1D, non-treated db/db mice exhibited a significant decrease in plasma adiponectin levels, compared with age-matched lean mice (db/+) (5444.04 ± 340.93 vs 8226.5 ± 674.08 ng/ml, $p < 0.05$). However, the plasma adiponectin levels significantly increased in the db/db mice treated with IBM-BMT + TT, compared with non-treated db/db mice (7437.5 ± 837.27 vs 5444.04 ± 340.93 ng/ml, $p < 0.05$) (Fig. 1D). The plasma insulin levels were higher in non-treated db/db mice than lean mice (116.7 ± 22.74 vs 45.0 ± 8.77 , $p < 0.05$), and decreased significantly after IBM-BMT + TT (67.2 ± 11.9 , $p < 0.05$) (Fig. 1E). In addition, the plasma IL-6 and IL-1 β levels significantly decreased in the db/db mice treated with IBM-BMT + TT (5 ± 1.2 and 4.16 ± 1.7 pg/ml, $p < 0.01$), compared with non-treated db/db mice (48.6 ± 1.7 and 23.5 ± 4.7 pg/ml, $p < 0.01$) (Fig. 1F and G). However, there were no significant differences in the levels of TNF α between the treated db/db and non-treated db/db mice (data not shown).

3.2. Morphology of pancreas, visceral fat, and liver

In the HE staining, non-treated db/db mice (Fig. 2B and E) showed larger islets and larger adipocytes in the visceral adipose tissue than lean mice (Fig. 2A and D). In contrast, smaller islets were noted in the db/db mice treated with IBM-BMT + TT (Fig. 2C and F) than non-treated db/db mice: The adipocytes were significantly larger in non-treated db/db mice than in lean mice (140.4 ± 8.17 vs 87.9 ± 7.52 μ m in diameter, $p < 0.01$), but smaller in the db/db mice treated with IBM-BMT + TT than in non-treated db/db mice (106.0 ± 1.29 vs 140.4 ± 8.17 μ m in diameter, $p < 0.01$). In addition, enlarged hepatocytes were found in non-treated db/db mice (Fig. 2H), although the hepatocytes in the db/db mice treated with IBM-BMT + TT (Fig. 2I) were similar in size to those in lean mice (Fig. 2G). Glycogen deposits were seen in the hepatocytes in all groups (Fig. 2J–L) by PAS reaction, and disappeared after diastase digestion (Fig. 2M–O). However, the density was lower in non-treated db/db mice than lean mice and the db/db mice treated with IBM-BMT + TT (Fig. 2I and L), suggesting that the impaired glycogen synthesis resulted from impaired insulin sensitivity in the non-treated db/db mice. Thus, the glycogen synthesis was also improved after IBM-BMT + TT.

3.3. Insulin content of pancreas islet

Insulin content (brown color) was much lower in the larger islets of non-treated db/db mice (Fig. 3B) than in those of lean mice (Fig. 3A), suggesting that much more insulin was secreted into the peripheral blood in the lean mice. However, there was significantly greater insulin content in residual beta cells (arrows in Fig. 3C) in the db/db mice treated with IBM-BMT + TT than the non-treated db/db mice. This suggests that beta cell destruction due to the exhaustion could be prevented by IBM-BMT + TT.

3.3.1. Effects of IBM-BMT + TT on insulin receptor phosphorylation and its signaling pathway—Visceral fat deposits drain into the portal circulation, resulting in the elevation of free fatty acids. This has been implicated in the genesis of impaired insulin

signaling and decreased phosphorylation of insulin receptors. We therefore examined the effects of IBM-BMT + TT on pancreatic tissue insulin receptor phosphorylation in the db/db mice treated with IBM-BMT + TT, non-treated db/db mice, and lean mice. The insulin receptor is a heterodimeric receptor tyrosine kinase with an extracellular alpha-chain, a transmembrane domain and an intracellular beta-chain. Additional autophosphorylation sites such as tyrosine residues 972 regulate the assembly of signal transduction complexes. Phosphorylation of insulin receptors at sites 972 was examined. As shown in Fig. 4A and B, insulin receptor phosphorylation at sites 972 significantly decreased in non-treated db/db mice, compared with lean mice. Densitometry analyses showed increases in the ratios of P-Tyr 972 in the db/db mice treated with IBM-BMT + TT, compared with non-treated db/db mice ($p < 0.03$); the levels were almost the same as those in lean mice. Similar results were observed in HO-1, HO-2, pAKT, pAMPK and pLKB1 expression (Fig. 4C–J, $p < 0.05$ vs IBM-BMT + TT).

3.4. Lymphocyte subpopulations (CD4/CD8 ratios) in thymus and in peripheral blood and donor-derived cells and lymphocyte function

Fig. 5A and B show lymphocyte populations in the thymus analyzed by FACS. The percentages of CD4⁺CD8⁺ double-positive cells decreased significantly in non-treated db/db mice, compared with lean mice ($74.6 \pm 2.04\%$ vs $84.5 \pm 0.22\%$, $p < 0.01$), while the percentages of CD4⁺CD8[−] double-negative cells increased significantly ($8.0 \pm 0.87\%$ vs $4.0 \pm 0.23\%$, $p < 0.01$). The percentages of CD4-positive cells more significantly increased in non-treated db/db mice than lean mice ($12.7 \pm 1.73\%$ vs $8 \pm 0.25\%$, $p < 0.05$). The total cell numbers of the thymus decreased significantly in non-treated db/db mice, compared with lean mice (7.8 ± 0.87 vs $12 \pm 0.77 \times 10^7$ cells, $p < 0.01$) (Fig. 5C). However, the cell numbers of the thymus increased significantly in the mice treated with IBM-BMT + TT, compared with non-treated db/db mice (11.8 ± 1.65 vs $7.8 \pm 0.87 \times 10^7$ cells, $p < 0.05$). Fig. 5D shows that the percentages of CD8-positive cells increased significantly in the db/db mice treated with IBM-BMT + TT, compared with non-treated db/db mice (9.5 ± 1.28 vs $4.76 \pm 0.74\%$, $p < 0.05$), while there was no significant difference in the percentages of CD4-positive cells (11.1 ± 1.95 vs $12.7 \pm 1.73\%$, ND).

Fig. 5E shows the ratios of CD4/CD8-positive cells in the peripheral blood. The ratio was significantly higher in non-treated db/db mice, compared with lean mice (2.16 ± 0.11 vs 1.3 ± 0.16 , $p < 0.05$), whereas it was significantly lower in the db/db mice treated with IBM-BMT plus TT, compared with non-treated db/db mice (1.25 ± 0.08 vs 2.16 ± 0.11 , $p < 0.01$). There was no significant difference in the ratio between lean mice and the db/db mice treated with IBM-BMT + TT.

Approximately, 98% of hematolymphoid cells were of donor-origin in the peripheral blood of the recipients treated with IBM-BMT + TT one month after BMT. Fig. 5F shows the results of analyses of cell surface antigens (CD4, CD8, B220 and CD11b) on donor-derived cells (15.80%, 11.90%, 42.79% and 21.29% of donor-derived cells) in the recipient mice; donor-derived cells with mature lineage markers were clearly observed one month after the treatment with IBM-BMT + TT.

The spleen cells of the recipients showed sufficient mitogen responses to both C on A and lipopolysaccharide (LPS) in comparison with those of non-treated db/db mice: 27254.9 ± 5558.15 vs 586.17 ± 51.85 ; 26416.2 ± 4164.60 vs 586.17 ± 51.85 , both $p < 0.001$. These findings suggest that not only T cell but also B cell functions were restored in the db/db mice treated with IBM-BMT + TT.

4. Discussion

Leptin is an adipocyte-derived hormone that links nutritional status with neuroendocrine and immune functions. Leptin has been shown to modulate T cell proliferation, to promote Th1 responses, and to protect thymocytes from corticosteroid-induced apoptosis *in vitro* [26–29]. Leptin-deficient ob/ob mice and leptin receptor-deficient db/db mice exhibit severe hereditary obesity [30,31] and display hormonal imbalances and hematolymphoid defects [32,33]. Db/db mice exhibit a marked reduction in the size and cellularity of the thymus [34,35]. The long-signaling leptin receptor isoform is expressed in the bone marrow cells, CD34 cells, marrow stroma cells, and both CD4 and CD8T cells of normal mice [26,27,36,37]; several investigators have described the direct effects of leptin on lymphocytes. It is, however, uncertain whether high blood glucose levels result from the imbalance of lymphocyte subsets in db/db mice.

Young patients with T2 DM show evidence of islet-cell auto-immunity, with autoantibodies present in 10–75% of patients, such as islet-cell antibodies (ICA) in 5–8%, glutamic acid decarboxylase (GAD) in 8–30%, islet-autoantibodies (IA) -2 in 8–42% and insulin antibodies in 5–35% [38–42]. These patients may be the evidence of islet autoimmunity contributing to insulin deficiency [43].

We previously showed that BMT could be used to treat non-insulin-dependent-diabetes in KK-Ay mice [8]. Recently, we have found that IBM-BMT treatment leads to increased HO-1 expression, resulting in preventing the development of T2 DM in ob/ob mice [13]. In the present study, we have shown that improved hyperglycemia and insulinemia result from normalizing the imbalance of the lymphocyte subsets in the db/db mice treated with IBM-BMT + TT. The db/db mice treated with IBM-BMT alone showed decreased blood glucose levels (150 mg/dl) one week after IBM-BMT, but increased blood glucose levels (300 mg/dl) two weeks after the treatment. In contrast, the db/db mice treated with IBM-BMT + TT showed normal blood glucose levels even 7 weeks after the treatment. These data are inconsistent with a previous report: Cruzado JM et al. reported that db/db mice treated with BMT alone showed normoglycemia even 10 weeks after the treatment [44].

In the present study, the db/db mice treated with IBM-BMT+ TT showed normalization of the percentages of DP, DN and CD4 cells in the thymus and also the normalization of CD4/CD8 ratios in the PB, which resulted in decreased plasma IL-6 and IL-1 β levels, and increased plasma adiponectin levels, followed by improved insulin sensitivity, and improved expression of AKT and pAKT on the liver and pancreas after IBM-BMT + TT. IBM-BMT + TT also led to the increased expression of HO-1 and pLKB1. The remarkable action of IBM-BMT + TT on adiponectin was associated with significant increases in pAKT and pAMPK expression, and also in the phosphorylation of insulin receptors; HO-1 increases the

production of adiponectin, resulting in enhanced pLKB1-AKT-AMPK crosstalk. Thus, it seems likely that IBM-BMT + TT is a strategy that could potentially be therapeutically employed for diabetes mellitus and metabolic syndrome.

It is well known that obesity is accompanied by chronic low-grade inflammation of adipose tissue, which increases the production of inflammatory cytokines such as leptin, TNF- α , chemoattractant protein-1 (MCP-1) and IL-6 [45]. In addition, it has been shown that there are nutritional treatments that are potentially capable of modulating insulin resistance and inflammation. DM induces a variety of metabolic abnormalities because of insufficient insulin action. Abnormalities in glucose metabolism are manifested clinically as hyperglycemia after glucose ingestion. Hyperglycemia produces oxidative stress through elevated levels of ROS, which leads to beta cell damage and vascular dysfunction through a variety of mechanisms [46–48]. The normal phenotype should differ from the state of overwork when beta cells compensate for insulin resistance to keep glucose levels normal. When only mild hyperglycemia develops, beta cells are subjected to glucotoxicity. As hyperglycemia becomes more severe, so does glucotoxicity [49]. The hyperglycemic state leads to overworking of the pancreatic beta cells and, in the long term, hyperglycemia induces glucotoxicity and worsening of the impaired insulin secretion. The glucotoxicity-mediated pancreatic beta cell dysfunction is reversible to some degree [50,51].

Autologous bone marrow-derived rat MSCs i) promote PDX-1 and insulin expression in the islets, ii) alter T cell cytokine patterns, iii) preserve regulatory T cells in the PB and iv) induce sustained non-moglycemia [52]. Bone marrow MSCs are self-renewing cells with the ability to differentiate into osteoblasts, chondrocytes and adipocytes under appropriate cell culture conditions. They can also differentiate into endothelial cells, hepatocytes and insulin-positive cells [53–55]. However, it remains to be elucidated how donor-derived MSCs can protect beta cells or can differentiate into beta cells.

Leptin injection induces a loss of bone marrow adipocytes and increases bone formation in leptin-deficient ob/ob mice [56]. In the present study, we have shown that fewer adipocytes are found in the bone of the db/db mice treated with IBM-BMT + TT than non-treated db/db mice. Progressive diabetic nephropathy in db/db mice was associated with increased numbers of kidney macrophages. Macrophage accumulation and activation correlated with prolonged hyperglycemia, glomerular immune complex deposition, and increased kidney chemokine production [57,58]. We have also found that more glycogen deposits are observed in the glomeruli in non-treated db/db mice than in lean mice, and that the deposits are improved as a result of IBM-BMT + TT (manuscripts in preparation). We are in the process of elucidating the exact mechanisms underlying these phenomena.

In conclusion, this is the first report indicating that increased insulin sensitivity and decreased blood glucose levels result from the normalized balance of lymphocyte subsets after IBM-BMT + TT in db/db mice. The novel effects of IBM-BMT + TT are that the treatment induces adiponectin secretion, followed by enhanced pLKB1-AKT-AMPK crosstalk, signaling pathway, insulin phosphorylation, and also HO-1. IBM-BMT + TT is a potential therapeutic intervention for metabolic disorders such as T2 DM, insulin-resistant diabetes and metabolic syndrome.

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Fig. 1.

Body weight, blood glucose levels, insulin sensitivity, and plasma adiponectin and insulin levels 7 weeks after the treatment (at the age of 13 weeks). (A) Body weights are shown. ($*p < 0.05$). (B) Fasting blood glucose levels ($*p < 0.001$). (C) Blood glucose levels after insulin administration ($*p < 0.05$). (D) Plasma adiponectin levels ($\#$, $*p < 0.05$). (E) Plasma insulin levels ($\#$, $*p < 0.05$). (F) Plasma IL-6 levels ($\#$, $*p < 0.01$). (g) Plasma IL-1 β levels ($\#$, $*p < 0.01$). The results are mean $\pm$ SE, $n = 6$ in each group.

Fig. 2.

The morphology of pancreas, visceral fat and liver. HE staining of the pancreas (A–C), visceral fat (E–F), and hepatocytes (G–I). Glycogen deposits in the hepatocytes (J–L) by PAS reaction, after diastase digestion (M–O).

Fig. 3.

Expression of insulin on the pancreata. Immunohistochemistry staining for insulin was performed (A–C). There was considerably more insulin content in residual beta cells (arrows in C) when compared to non-treated db/db mice.

Fig. 4. Effect of IBM-BMT + TT on insulin receptor phosphorylation and its signaling pathway. (A) Western blot and densitometry analyses of insulin receptor phosphorylation (Tyr 972) and actin proteins in pancreata of lean, non-treated db/db mice and db/db mice treated with IBM-BMT + TT. (B) The ratio of quantitative densitometry evaluation of p-Tyr 972 and actin proteins was determined. Representative immunoblots are shown ($n = 3$). The expression of HO-1, HO-2, pAKT, pAMPK and pLKB1 are shown (C,E,G, I). Their ratios are shown ($p < 0.05$ vs IBM-BMT + TT) (D, F, H, J).

Fig. 5.

Lymphocyte populations in the thymus and the ratios of CD4/CD8 in the peripheral blood and donor-derived cells. (A,B) Lymphocyte populations in the thymus by FACS, * $p < 0.01$, # $p < 0.01$. (C) Total numbers of thymocytes * $p < 0.01$, # $p < 0.05$. (D) Percentages of CD8-positive cells. # $p < 0.05$, $n = 6$ in each group. (E) The ratios of CD4/CD8-positive cells in the peripheral blood. * $p < 0.05$, # $p < 0.01$. The results are mean $\pm$ SE, $n = 6$ in each group. (F) Analyses of CD4, CD8, B220 and CD11b on donor-derived cells in recipient mice one month after IBM-BMT + TT.