Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 328 (2005) 318–325
BBRC
www.elsevier.com/locate/ybbrc

Reversal of experimental diabetes by multiple bone marrow transplantation☆

Meenal Banerjee[a], Anil Kumar[b], Ramesh R. Bhonde[a,*]

[a] National Centre for Cell Science, Ganeshkhind, Pune, Maharashtra, India
[b] School of Biotechnology, Devi Ahilya University, Indore, Madhya Pradesh, India

Received 4 December 2004
Available online 7 January 2005

Abstract

Therapeutic utility of bone marrow transplantation in diabetic patients to overcome deficient β-cell population is an attractive proposal. However, the status of bone marrow stem cells (BMSCs) under hyperglycemia is not known. In the present study, we investigated the status of BMSCs in experimental-diabetic mice and demonstrated the rescue of experimental diabetes by multiple diabetic bone marrow transplantation. Our flow-cytometry analysis for $CD34^+$, $CD45^+$, $flk1^+$, c-kit^+, and $CD34^+CD45^+$ revealed that BMSC reserve remains unaffected under sustained hyperglycemia. We found that single injection of diabetic bone marrow cells ($\sim 10^6$) resulted in reduction and stabilization of moderate hyperglycemia. However, multiple injections at regular intervals led to restoration of stabilized normoglycemia during a 30 day follow-up. Reversal of diabetes was evidenced by disappearance of hyperglycemia, normal intra-peritoneal glucose tolerance test, and histology and morphometry of pancreas. The present study thus demonstrates that diabetic bone marrow retains its stemness and potential to induce pancreatic regeneration on transplantation.

Keywords: Diabetic bone marrow; Pancreatic regeneration; Hematopoietic stem cell; Hyperglycemia; Stemness; Experimental diabetes

A reduction of β cell mass in the pancreas is a hallmark in the development of both type1 (IDDM) and type 2 diabetes (NIDDM) that need to be compensated for better glycemic control. The regeneration and maintenance of pancreatic endocrine tissue after the onset of islet destruction would have significant therapeutic impact on diabetes mellitus (DM). Regeneration of pancreatic endocrine cells/islets has been demonstrated on several occasions including the work from this laboratory [1–6]. Recent animal and clinical studies have suggested that tissue regeneration can occur upon the introduction of bone marrow derived stem cells that have multiple effects. It seems possible that pleuripotent stem cells derived from the bone marrow contribute to adult islet neogenesis [7]. Bone marrow harbors cells that have pleuripotent differentiation capacity since myoblasts [8], cardiac myoblasts [9], endothelium [10], hepatic, biliary duct epithelium [11], and neuroectodermal cells [12] of donor origin can be found in recipient animals upon transplantation of bone marrow or enriched hematopoietic stem cells (HSCs). It is possible that HSCs may also be able to switch lineage and therefore, be a convenient source of stem cells for both inducing tolerance and islet cell regeneration [13].

Recently, Ianus et al. [14] have reported that bone marrow cells can differentiate into functionally competent pancreatic endocrine β cells and thus represent a source for cell-based treatment for DM. On other hand,

☆ *Abbreviations:* BSA, bovine serum albumin; FACS, fluorescence-activated cell sorter; HSC, hematopoietic stem cell; PBS, phosphate-buffered saline; STZ, streptozotocin.

* Corresponding author. Fax: +91 20 25692259.
E-mail address: rrbhonde@nccs.res.in (R.R. Bhonde).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.12.176

studies of Watada and colleagues suggested that bone marrow derived cells are a distinct cell population from islet cells and that transdifferentiation from bone marrow derived cells to pancreatic β cell is a rare event [15,16]. While determining the cellular mechanism and physiological relevance of bone marrow derived stem cells for the restoration of tissue function after pancreatic injury, Hess et al. [17] have introduced a new concept to the field of bone marrow stem cell research. They have shown that upon transplantation these cells initiate endogenous pancreatic regeneration by rapid proliferation of recipient pancreatic cells and neogenesis of insulin positive cells of recipient origin.

Slack [18] has mentioned that the failure of regeneration following treatment with streptozotocin (STZ) or alloxan may suggest that these drugs target the potential stem or transit cells as well as the differentiated β cells. Contrary to this report, we and other groups have shown that STZ does not destroy the intra-islet stem cell reserve in experimental-diabetic subjects [19,6]. Hence, we were interested in investigating the status of bone marrow stem cells in STZ-diabetic mice with respect to their regeneration inducing capacity. Stepanovic et al. [20] have reported that exogenous non-diabetic bone marrow derived cells increase vascularization and improve wound healing in Leprdb-diabetic mice whereas Leprdb derived bone marrow cells inhibit vascularization but promote wound healing in Leprdb mice, thus indicating retention of stemness, despite hyperglycemia. In recent years, evidence is being accumulated regarding the role of bone marrow transplantation in induction of pancreatic regeneration in diabetic animals. However, the stemness of bone marrow cells under the influence of sustained hyperglycemia is questionable. Hence, we isolated bone marrow from STZ-diabetic mice and compared it with that of normal mice with respect to HSCs as well as endothelial stem cell population. CD34 is an *O*-sialylated glycoprotein (105–120 kDa), whose expression within the hematopoietic system is restricted to primitive progenitor cells of all lineages [21] and CD45, a pan-hematopoietic stem cell marker which represents only hematopoietic stem cell population, neither mesenchymal nor multipotential adult progenitor stem cells [22]. Several studies have demonstrated that endodermal interaction with endothelial cells [23] is necessary for pancreatic organogenesis. In addition, endothelial cells, another mesodermal derivative, provide organogenic stimuli for the pancreas [24]. *Flk1* encodes a receptor for VEGFs and is expressed in endothelial cells [25]. On assessing the integrity of hematopoietic and endothelial stem cell population in diabetic bone marrow, we transplanted whole bone marrow of STZ-diabetic mice into syngenic experimental-diabetic mice and looked for changes in glycemic status, morphometry, and histology of pancreatic sections to confirm regeneration if any.

Materials and methods

Animals. STZ treated as well as normal Swiss albino mice (6–8 weeks) were maintained in the animal house of National Centre for Cell Science on normal chow and water. Experimental protocols were approved by Animal Ethical Committee of the institute.

Antibodies. Normal goat IgG, normal rabbit IgG, CD45 (goat polyclonal IgG), CD34 (rabbit polyclonal IgG), c-kit (rabbit polyclonal IgG), (Flk-1 goat polyclonal IgG), goat anti-rabbit IgG-FITC, and donkey anti-goat IgG-PE, were all purchased from Santa Cruz Biotechnology, Santa Cruz, CA, USA.

Induction of experimental diabetes by STZ. After overnight fasting mice were injected with freshly prepared STZ (Sigma, St. Louis, MO, USA, 200 mg/kg body weight, prepared in chilled citrate buffer, pH 4.5). Diabetic status was confirmed by measurement of blood glucose (Accutrend sensor comfort blood glucose meter, Roche Diagnostic, Germany) after one week of STZ injection. Glycosylated hemoglobin levels of STZ-diabetic mice were also analyzed. Mice exhibiting glycosylated hemoglobin more than 6.6 were taken as donors for transplantation studies.

Bone marrow cell isolation and transplantation. Bone marrow from STZ-diabetic mice was flushed from the medullary cavities of femurs using a 25 gauge needle. Viability of cells after isolation was checked by Trypan blue (Sigma, St. Louis, MO, USA) dye exclusion method. Recipient mice (diabetic, irradiated/non-irradiated) were transplanted intravenously through tail vein approximately 10^6 unfractionated bone marrow cells either as a single injection or multiple injections (at least three unless otherwise specified) at a regular interval of 6 days.

Intraperitoneal glucose tolerance test. After fasting overnight all three groups of mice, i.e., normal, diabetic, and experimental (which received multiple bone marrow injections) were injected with 2 g/kg body weight of glucose intraperitoneally. Glucose disposal was analyzed by measuring random blood glucose (Accutrend sensor comfort blood glucose meter, Roche Diagnostic, Germany) of mice at different time points 0, 30, 60, and 120 min.

Total body irradiation. In order to destroy the stem cell niche of bone marrow of the recipient STZ-diabetic mice, they were subjected to total body irradiation with a dose of 900 rads from a ^{60}Co source.

Histological and morphometry analysis. Pancreata were excised, washed once in phosphate-buffered saline (PBS), and then fixed in formalin (10%). Tissues were then processed for paraffin embedding, subsequent serial sectioning, and stained with hematoxylin/eosin (Qualigens Fine Chemicals, Bombay, India) to allow the assessment of pancreatic islet morphology before and after transplantation. Islets were observed from every tenth serial section, images were captured with a Variocam, Germany PCO CCD imaging camera, processed, and then quantification of islet size was carried out using the Kontron Image analysis software version 2.04. Values are presented as means ± SE.

Immunostaining of bone marrow hematopoietic as well as endothelial stem cells for flow cytometry. Whole bone marrow was retrieved from STZ-diabetic mice after one month of sustained hyperglycemia as well as from age- and sex-matched normal mice following the protocol mentioned above. Cells were seeded in 25 cm^2 tissue culture flasks with IMDM (Iscove's modified Dulbecco's medium, Grand Island, NY, USA) supplemented with 20% fetal calf serum (Trace Biosciences PTY, New South Wales, Australia). Flasks were incubated for 2 h at 37 °C in 5% CO_2 atmosphere. Bone marrow cells isolated from normal as well as diabetic mice were divided into three groups: unstained, isotype control, and experimental. Cells were then washed twice with PBS, pH 7.4 and fixed with 2% paraformaldehyde at 4 °C for 10 min (for CD34 and Flk1) or with chilled methanol (CD45 and c-kit staining). Experimental group of cells was washed with PBS twice and then incubated with respective primary antibody (5 μg/ml) at 4 °C for 2 h with occasional shaking. Unstained group of cells was incubated with only PBS and isotype control cells were incubated with normal goat

IgG, 5 μg/ml or with normal rabbit IgG, 5 μg/ml. After two washes with PBS containing 1% BSA (bovine serum albumin), experimental as well as isotype control cells were incubated with secondary antibody (donkey anti-goat IgG-PE 5 μg/ml or goat anti-rabbit IgG-FITC 5 μg/ml) at 4 °C for 1 h with occasional shaking. Cells were washed twice with PBS containing 1% BSA, once with PBS only and analyzed immediately on FACS Vantage (Becton–Dickinson, Canada).

Immunostaining for co-expression of CD34 and CD45 of bone marrow HSCs for flow cytometry. Bone marrow cells were isolated, cultured, and grouped according to the protocol mentioned above. After the fixation of cells with paraformaldehyde they were incubated with CD34 antibody (5 μg/ml) at 4 °C for 2 h with occasional shaking. Cells were then permeabilized with 0.1% Triton X-100 for 2 min at 4 °C. After two washes with PBS containing 1% BSA, experimental cells were incubated with CD45 (5 μg/ml) antibody at 4 °C for 2 h which is followed by secondary antibody incubation at 4 °C for 1 h (donkey anti-goat IgG-PE 5 μg/ml or goat anti-rabbit IgG-FITC 5 μg/ml, both added simultaneously). Unstained and isotype control groups of cells were treated as mentioned earlier. Cells were washed twice with PBS and analyzed immediately on FACS Vantage (Becton–Dickinson, Canada).

Statistical analysis. Data have been presented as means ± SE. Statistical differences were determined using a one-way ANOVA (Tukey test). Analysis was performed with Sigma Stat; Jandel Scientific, Erkrath, Germany. A value of $P < 0.05$ was considered to be statistically significant.

Results

Comparative analysis of bone marrow HSCs as well as endothelial stem cells expressing CD34, CD45, c-kit, and Flk1

Comparative quantitative analysis for the existence of bone marrow HSCs isolated from normal as well as STZ-diabetic mice was performed by flow cytometry. Data (Fig. 1) presented here clearly show that there is no significant difference between percent population of bone marrow HSCs expressing either CD45 (Fig. 1A) or CD34 (Fig. 1B), HSC subpopulation expressing c-kit (Fig. 1C) and endothelial stem cells expressing Flk1 (Fig. 1D) in both normal as well as diabetic groups.

Comparative analysis of bone marrow HSCs co-expressing CD34 and CD45

Scatter plot (Figs. 2A and B) obtained for normal as well as diabetic mice bone marrow exhibited no significant differences in cell population of normal as well as diabetic bone marrow. Figs. 2C and D demonstrates that the co-expression pattern of HSC population (HSCs co-expressing CD34 and CD45) remains unaffected under severe hyperglycemic shock.

Effect of bone marrow transplantation on hyperglycemic status of experimental-diabetic mice

The effect of single and multiple injections of bone marrow at an interval of 6 days has been described in (Fig. 3). Single bone marrow injection reduced blood glucose and prevented further rise in blood glucose level in experimental-diabetic mice however it failed to restore normoglycemia. On the other hand, multiple bone marrow injections led to reduction in hyperglycemia culminating in restoration of normoglycemia which remained steady during the course of experiment without further relapse.

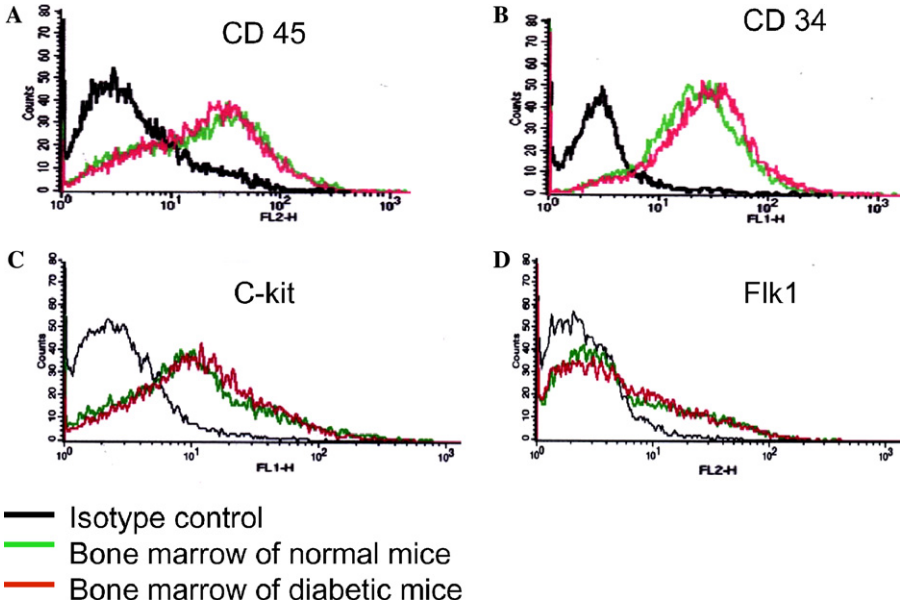

Fig. 1. Comparative analysis of normal and diabetic bone marrow stem cells by FACS. $CD45^+$ hematopoietic stem cells (A), $CD34^+$ hematopoietic stem cells (B), c-kit positive subpopulation of hematopoietic stem cells (C), and Flk1 positive endothelial stem cells (D) of bone marrow derived from normal and STZ-diabetic mice.

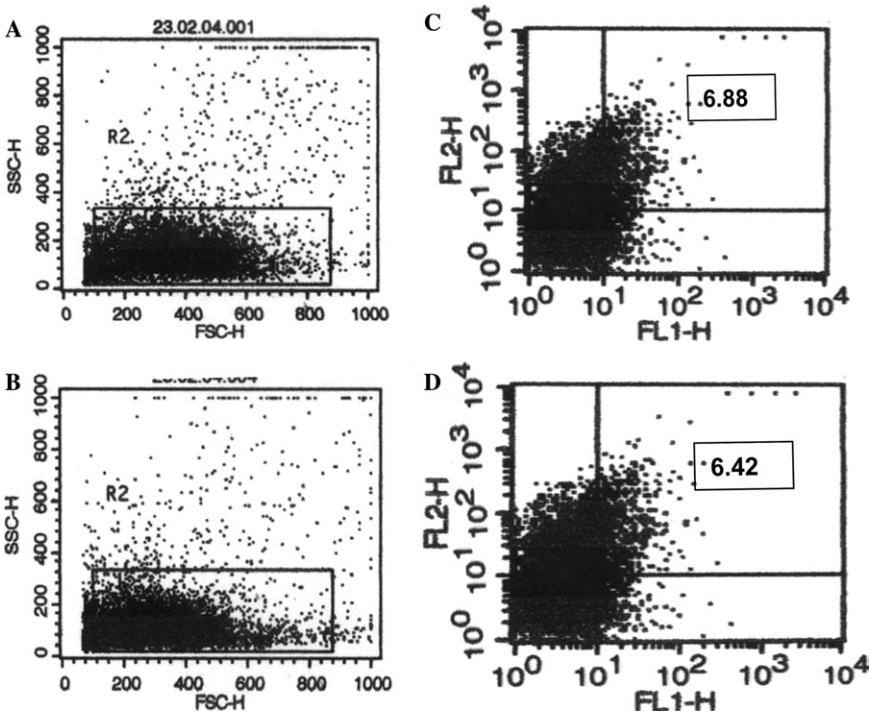

Fig. 2. Comparative analysis of CD34 and CD45 dual positive bone marrow stem cells of normal and diabetic mice. Scatter plot of bone marrow cells of normal mice (A) and diabetic mice (B). Percentage of dual positive (CD34-FL1 and CD45-FL2) bone marrow hematopoietic stem cells of normal (C) and diabetic mice (D). Number in square boxes are representative of percentage of dual positive cell population.

A

Injections ⇩	Number of Days ⇨ 0	6	12	18	30
Single (Normal)	398.25 ±11.3	321.38 ± 9.4	320.13 ± 10.1	316.75 ± 9.7	314.75 ± 11.3
Multiple (Normal)	371.13 ± 8.6	316.75 ± 9.7	187.88 ± 5.7	128.25 ± 3.2	120.50 ± 1.6
Single (Diabetic)	460.12 ± 12.5	364.25 ± 11.7	331.87 ± 8.8	319.62 ± 3.9	321.87 ± 4.1
Multiple (Diabetic)	453.62 ± 10.6	350.50 ± 7.5	213.50 ± 7.1	148.25 ± 6.2	132.50 ± 3.0

B

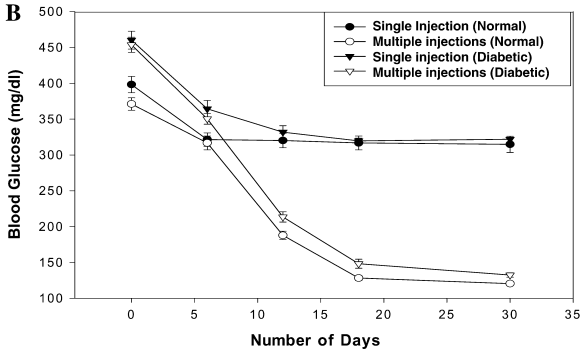

Fig. 3. Effect of whole bone marrow transplantation on blood glucose level. Tabular representation of single and multiple injections of normal as well as diabetic whole bone marrow (A), graphical representation of above (B).

Effect of bone marrow transplantation in irradiated recipient-diabetic mice

STZ treated mice were sublethally irradiated and used as recipients. Experimental group of mice was transplanted intravenously with bone marrow cells whereas control mice received intravenous injection of PBS only. Reduction in hyperglycemia as well as increased survival was found among experimental group of mice whereas control group of mice remained hyperglycemic throughout the experiment and the survival rate was low, i.e., 50% (data not shown).

Effect of bone marrow transplantation on glucose disposal capacity of hyperglycemic recipients

The reversal of experimental diabetes in mice with multiple bone marrow injections was characterized by intra-peritoneal glucose tolerance test. It was observed that diabetic mice remained glucose intolerant throughout the experiment whereas experimental mice (which received multiple bone marrow injections) improved tolerance to glucose and showed a glucose tolerance curve similar to that of control mice (Fig. 4A).

Effect of bone marrow transplantation on the induction of pancreatic regeneration

Histological examination of the hematoxylin and eosin stained paraffin sections of mouse pancreas subjected to multiple bone marrow injections suggested that STZ-diabetic mice bone marrow cells initiate pancreatic regeneration upon transplantation into recipient experimental-diabetic mice. Pancreatic regeneration was evidenced by the appearance of many small islets (Fig. 4B). Many of these small islets were found near the duct which is suggestive of new islet formation.

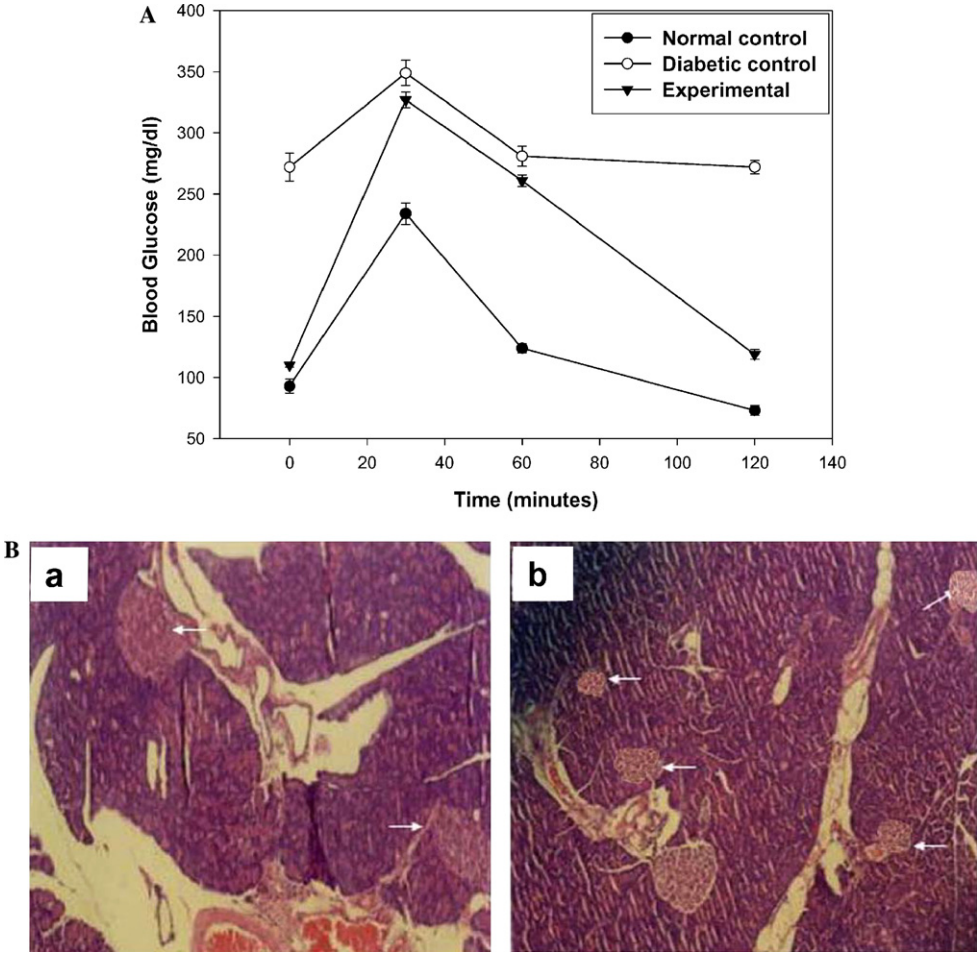

Fig. 4. (A) Glucose tolerance test for examination of glucose clearance pattern. Graph represents glucose disposal curves of normal control, diabetic control, and experimental (multiple bone marrow injected) mice. All three groups of mice overnight fasted and then injected with 2 gm/kg body weight of glucose intraperitoneally. Blood glucose level was measured at different time intervals of 0, 30, 60, and 120 min after glucose injection. Values are presented as means ± SE when $P < 0.001$. (B) Histopathological analysis of pancreatic sections. Hematoxylin and eosin stained pancreatic sections of control and experimental mice. Arrows representing normal mouse pancreatic islets (a), arrows representing many small islets (b), which are significantly smaller compared to those of control islets in the pancreas of experimental mice (mice subjected to multiple injections of bone marrow), 200×.

Islet image analysis

Image analysis of control as well as newly generated islets has been performed with paraffin sections of pancreas. Islet mean area of control islets measured was $16456.1 \pm 1612.5\ \mu m^2$ whereas the mean area of newly generated islets was $1440.4 \pm 162.3\ \mu m^2$. Mean diameter of control islets measured was $121.9 \pm 6.1\ \mu m$ whereas for newly generated islets it was $39.3 \pm 2.5\ \mu m$. Thus, the values for islet area as well as diameter of control islets were found to be significantly higher than those of newly generated islets.

Discussion

The purpose of the present study was to evaluate the status of bone marrow hematopoietic and endothelial stem cells under normoglycemic and sustained hyperglycemic conditions (glycosylated hemoglobin level >6.6) and also their capacity to induce pancreatic regeneration so as to compensate the loss of β cells upon transplantation in the recipient experimental-diabetic mice. On confirming the ability of normal non-diabetic bone marrow transplantation to restore normoglycemia in experimental-diabetic mice (Fig. 3), we characterized the stem cell population within normal and diabetic bone marrow. For this purpose, we compared by flow cytometry the percentage of normal as well as diabetic bone marrow HSC population expressing cell surface markers either CD34 or CD45 alone and CD34 and CD45 simultaneously. Our data (Figs. 1 and 2) showed that the bone marrow hematopoietic as well as endothelial stem cell reserve in terms of the percentage of CD34, CD45, c-kit, and Flk1 population remained intact under sustained hyperglycemic status. This is perhaps the first report for the existence of bone marrow stem cell population within a diabetic subject which is of highly therapeutic significance. These data prompted us to investigate whether transplantation of such diabetic bone marrow into syngenic-diabetic mice leads to restoration of normoglycemia as an intervening step to induce regeneration. To serve this purpose we transplanted into recipient diabetic mice the whole unfractionated bone marrow instead of taking only CD34 and CD45 HSCs or the subpopulation of HSCs from STZ-diabetic mice so as not to disturb the naturally existing stem cell niche within bone marrow microenvironment [26]. Injections of conditioned medium from diabetic bone marrow failed to restore normoglycemia in experimental-diabetic mice (unpublished data), indicating that soluble factors derived from bone marrow cells did not show any effect on blood glucose level. This finding is supported by the work of Li et al. [27].

Our results demonstrate that single injection of bone marrow although helping to reduce blood glucose level and preventing further blood glucose hike is however insufficient to restore normoglycemia. On the other hand, multiple injections (at least three) of bone marrow could achieve normoglycemia over a long period of time without requiring further injections, irrespective of the source of donor bone marrow. The success in restoration of stable normoglycemia which has not been reported earlier [17] goes to multiple doses of bone marrow transplantations that might have led to infusion of desired number of HSC population sufficient to compensate the loss or dysfunction of β cell mass occurring during diabetes. Although single injection of bone marrow failed to achieve normoglycemia, it certainly led to reduction of hyperglycemia which remained stable in subsequent follow-up (Fig. 3). This finding is equally important as it can permit long-term survival of experimental-diabetic animals without mortality which are warranted to undertake long-term studies. Mortality of drug/virus induced diabetic animals [28–30] has always been an obstacle to study complications of chronic hyperglycemia as it is difficult to control tailor-made loss of β cells. Our present study suggests an alternative approach for the modulation of experimental diabetes depending on the number of bone marrow injections to serve the purpose. This is perhaps the first report demonstrating modulation of experimental diabetes under controlled conditions with respect to the number of cells infused to suit the convenience of researchers to achieve the desired goal.

Our data provide sufficient evidence through glycemic status, glucose tolerance test, and histopathology and morphometry analysis for pancreatic regeneration caused in recipient mice due to repeated bone marrow transplantations. Glucose tolerance test confirmed normalization of blood glucose level in bone marrow transplanted mice (Fig. 4A). These mice remained normoglycemic, even after a 30 day follow-up without remission indicating complete reversal of experimental diabetes. Bone marrow transplantation into sublethally irradiated experimental-diabetic mice revealed that the donor bone marrow alone is contributing to reduction of blood glucose level of recipient mice (data not shown). Our histological (Fig. 4B) and morphometry analysis of pancreas of experimental mice has shown the appearance of many small islets throughout the sections [31]. Their mean diameter and area analysis revealed that they are significantly smaller compared to control islets depicting islet neogenesis. Thus, we claim that whole bone marrow derived from experimental-diabetic mice retain their potential to induce pancreatic regeneration upon transplantation in syngenic recipient-diabetic mice. Recently, few bone marrow transplantation studies in experimental-diabetic mice have established the fact that cells or factors derived from bone marrow play important supportive role either in inducing pancreatic regeneration [17] or in modifying

the viability of endocrine islet cells [27] indicating the possible mechanism. Recent studies of Tang et al. [32] have provided direct evidence supporting a notion that transdifferentiation of adult bone marrow stem cells to functional insulin producing cells may represent a viable therapeutic option for type 1 diabetes. Latest studies by Ende et al. [33] demonstrate potential human umbilical cord blood mononuclear cells in lowering the blood glucose level in non-obese diabetic (NOD) mice upon i.v. administration, further supporting our present data.

Though the exact fate of transplanted-diabetic bone marrow as well as the functionality of diabetic bone marrow stem cells in vitro remains to be seen, our finding is of clinical significance to advocate use of bone marrow transplantation as an alternative therapy to treat diabetes.

Acknowledgments

Authors acknowledge The Director, National Centre for Cell Science (NCCS) for support and encouragement to carry out the present work and also to Dr. Avinash Pradhan (Histopathologist, KEM hospital) for his expert comments on histopathological studies.

References

[1] S. Bonner-Weir, L.A. Baxter, G.T. Schuppin, F.E. Smith, A second pathway for regeneration of adult exocrine and endocrine pancreas: a possible recapitulation of embryonic development, Diabetes 42 (1993) 1715–1730.
[2] L. Rosenberg, In vivo cell transformation: neogenesis of beta cells from pancreatic ductal cells, Cell Transplant. 4 (1995) 371–383.
[3] A.A. Hardikar, M.S. Karandikar, R.R. Bhonde, Effect of partial pancreatectomy on diabetic status in BALB/c mice, J. Endocrinol. 162 (1999) 189–195.
[4] A.A. Hardikar, R.R. Bhonde, Modulating experimental-diabetes by treatment with cytosolic extract from the regenerating pancreas, Diabetes Res. Clin. Pract. 46 (1999) 203–211.
[5] M.V. Risbud, R.R. Bhonde, Models of pancreatic regeneration in diabetes, Diabetes Res. Clin. Pract. 58 (2002) 155–165.
[6] Y. Guz, I. Nasir, G. Teitelman, Regeneration of pancreatic beta cells from intra-islet precursor cells in an experimental model of diabetes, Endocrinology 142 (2001) 4956–4968.
[7] A. Lechner, J.F. Habener, Stem/progenitor cells derived from adult tissues: potential for the treatment of diabetes mellitus, Am. J. Physiol. Endocrinol. Metab. 284 (2003) 259–266.
[8] G. Ferrari, F. Mavilio, Myogenic stem cells from the bone marrow: a therapeutic alternative for muscular dystrophy, Neuromuscul. Disord. 12 (2002) 7–10.
[9] D. Orlic, J. Kajstura, S. Chimenti, I. Jakoniuk, S.M. Anderson, B. Li, J. Pickel, R. McKay, B. Nadal-Ginard, D.M. Bodine, A. Leri, P. Anversa, Bone marrow cells regenerate infarcted myocardium, Nature 410 (2001) 701–705.
[10] Y. Lin, D.J. Weisdorf, A. Solovey, R.P. Hebbel, Origins of circulating endothelial cells and endothelial outgrowth from blood, J. Clin. Invest. 105 (2000) 71–77.
[11] B.E. Petersen, W.C. Bowen, K.D. Patrene, W.M. Mars, A.K. Sullivan, N. Murase, S.S. Boggs, J.S. Greenberger, J.P. Goff, Bone marrow as a potential source of hepatic oval cells, Science 284 (1999) 1168–1170.
[12] T.R. Brazelton, F.M. Rossi, G.I. Keshet, H.M. Blau, From marrow to brain: expression of neuronal phenotypes in adult mice, Science 290 (2000) 1775–1779.
[13] R.K. Burt, Y. Oyama, A. Traynor, N.S. Kenyon, Hematopoietic stem cell therapy for type 1 diabetes: induction of tolerance and islet cell neogenesis, Autoimmun. Rev. 3 (2002) 133–138.
[14] A. Ianus, G.G. Holz, N.D. Theise, M.A. Hussain, In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion, J. Clin. Invest. 111 (2003) 843–850.
[15] J.B. Choi, H. Uchino, K. Azuma, N. Iwashita, Y. Tanaka, H. Mochizuki, M. Migita, T. Shimada, R. Kawamori, H. Watada, Little evidence of transdifferentiation of bone marrow-derived cells into pancreatic beta cells, Diabetologia 46 (2003) 1366–1374.
[16] A. Lechner, Y.G. Yang, R.A. Blacken, L. Wang, A.L. Nolan, J.F. Habener, No evidence for significant transdifferentiation of bone marrow into pancreatic β-cells in vivo, Diabetes 53 (2004) 616–623.
[17] D. Hess, L. Li, M. Martin, S. Sakano, D. Hill, B. Strutt, S. Thyssen, D.A. Gray, M. Bhatia, Bone marrow-derived stem cells initiate pancreatic regeneration, Nat. Biotechnol. 21 (2003) 763–770.
[18] J.M.W. Slack, Developmental biology of the pancreas, Development 121 (1995) 1569–1580.
[19] M. Banerjee, R.R. Bhonde, Islet generation from intra islet precursor cells of diabetic pancreas: in vitro studies depicting in vivo differentiation, JOP 4 (2003) 137–145.
[20] V. Stepanovic, O. Awad, C. Jiao, M. Dunnwald, G.C. Schatteman, Leprdb diabetic mouse bone marrow cells inhibit skin wound vascularization but promote wound healing, Circ. Res. 92 (2003) 1247–1253.
[21] D.R. Sutherland, A. Keating, The CD34 antigen: structure, biology and potential clinical applications, J. Hemother. 1 (1992) 115–129.
[22] F.D. Camargo, R. Green, Y. Capetenaki, K.A. Jackson, M.A. Goodell, Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates, Nat. Med. 9 (2003) 1520–1527.
[23] E. Lammert, O. Cleaver, D. Melton, Induction of pancreatic differentiation by signals from blood vessels, Science 294 (2001) 564–567.
[24] O. Cleaver, D.A. Melton, Endothelial signaling during development, Nat. Med. 9 (2003) 661–668.
[25] B. Millauer, S. Wizigmann-Voos, H. Schnurch, R. Martinez, N.P. Moller, W. Risau, A. Ullrich, High affinity VEGF binding and developmental expression suggests Flk-1 as a major regulator of vasculogenesis and angiogenesis, Cell 72 (1993) 835–846.
[26] J.F. Zhong, Y. Zhan, W.F. Anderson, Y. Zhao, Murine hematopoietic stem cell distribution and proliferation in ablated and nonablated bone marrow transplantation, Blood 15 (2002) 3521–3526.
[27] F.X. Li, J.W. Zhu, J.S. Tessem, J. Beilke, M. Varella-Garcia, J. Jensen, C.J. Hogan, J. DeGregori, The development of diabetes in *E2f1*/*E2f2* mutant mice reveals important roles for bone marrow-derived cells in preventing islet cell loss, Proc. Natl. Acad. Sci. USA 100 (2003) 12935–13940.
[28] S. Kurup, R.R. Bhonde, Combined effect of nicotinamide and STZ on diabetic status in partially pancreatectomized adult BALB/c mice, Horm. Metab. Res. 32 (2000) 330–334.
[29] R.R. Bhonde, Factors influencing induction and reversal of virus induced diabetes, Indian J. Exp. Biol. 35 (1997) 267–271.
[30] G.C. Weir, J.L. Leahy, S. Bonner-Weir, Experimental reduction of B-cell mass: implications for the pathogenesis of diabetes, Diabetes Metab. Rev. 2 (1986) 125–161.

[31] M. Vessal, M. Hemmati, M. Vasei, Antidiabetic effects of quercetin in streptozocin-induced diabetic rats, Comp. Biochem. Physiol. C Toxicol. Pharmacol. 3 (2003) 357–364.
[32] D.Q. Tang, L.Z. Cao, B.R. Burkhardt, C.Q. Xia, S.A. Litherland, M.A. Atkinson, L.J. Yang, In vivo and in vitro characterization of insulin-producing cells obtained from murine bone marrow, Diabetes 53 (2004) 1721–1732.
[33] N. Ende, R. Chen, A.S. Reddi, Effect of human umbilical cord blood cells on glycemia and insulitis in type 1 diabetic mice, Biochem. Biophys. Res. Commun. 325 (2004) 665–669.