Stem-cell therapy for diabetes mellitus

Mehboob A Hussain, Neil D Theise

Lancet 2004; 364: 203–05

Liver and Stem Cell Research Laboratory, Division of Digestive Diseases, Department of Internal Medicine, Beth Israel Medical Center, Albert Einstein College of Medicine, New York, NY 10003, USA (M A Hussain MD, N D Theise MD)

Correspondence to: Dr Mehboob Hussain
mehboobhussain@yahoo.com

Context **Curative therapy for diabetes mellitus mainly implies replacement of functional insulin-producing pancreatic β cells, with pancreas or islet-cell transplants. However, shortage of donor organs spurs research into alternative means of generating β cells from islet expansion, encapsulated islet xenografts, human islet cell-lines, and stem cells. Stem-cell therapy here implies the replacement of diseased or lost cells from progeny of pluripotent or multipotent cells. Both embryonic stem cells (derived from the inner cell mass of a blastocyst) and adult stem cells (found in the postnatal organism) have been used to generate surrogate β cells or otherwise restore β-cell functioning.**

Starting point **Recently, Andreas Lechner and colleagues failed to see transdifferentiation into pancreatic β cells after transplantation of bone-marrow cells into mice (*Diabetes* 2004; 53: 616–23). Last year, Jayaraj Rajagopal and colleagues failed to derive β cells from embryonic stem cells (*Science* 2003; 299: 363). However, others have seen such effects.**

Where next? **As in every emerging field in biology, early reports seem confusing and conflicting. Embryonic and adult stem cells are potential sources for β-cell replacement and merit further scientific investigation. Discrepancies between different results need to be reconciled. Fundamental processes in determining the differentiation pathways of stem cells remain to be elucidated, so that rigorous and reliable differentiation protocols can be established. Encouraging studies in rodent models may ultimately set the stage for large-animal studies and translational investigation.**

Embryonic stem cells (ESC) can be differentiated into insulin-producing cells by manipulating culture conditions. In-vitro differentiation of mouse ESC can generate embryoid bodies, which, after selection for nestin-expressing ESC, were stimulated to differentiate towards a β-cell-like phenotype.[1] The addition of phosphoinositide kinase inhibitors promoted differentiation of larger numbers of ESC towards functional β cells.[2] Variations in ESC-culture conditions generate cells with properties of β cells.[3–5] With manipulation of culture conditions and use of pax4 or pdx-1, transcription factors associated with β-cell lineage[6,7] yield promising results.

Some doubt has been cast on whether ESC differentiation protocols truly yield cells that produce insulin, or cells that merely absorb insulin from the medium.[8] These differentiated cells must actively synthesise and secrete insulin rather than insulin being detected. Functioning molecular components of regulated secretion of insulin and insulin-containing vesicles are additional features that indicate a β-cell phenotype. Transplantation of ESC-derived insulin-producing cells reverses diabetes in rodents,[2,6] indicating that these cells do synthesise and release insulin. The early and uncontrolled introduction of transcription factors into ESC during in-vitro differentiation might not yield the desired results.[5] Regulated expression of introduced transcription factors that can be turned on during in-vitro differentiation of ESC might be more successful.[7] ESC, genetically selected for insulin expression and injected into diabetic rats, improve glucose control.[10]

Human ESC produce insulin under different culture conditions.[11,12] Techniques that do not require murine feeder cells have been developed, allowing for single-species propagation of ESC and avoiding possible zoonotic infection of cells intended for clinical use.[13] Problems in control of differentiation and teratoma formation from ESC-derived insulin-producing cells remain to be overcome.[14] Existing ESC lines are not assumed to be identical or ideal for generating islets or β cells. Hence additional ESC lines continue to be generated.[15] Ethical concerns about the use of ESC need to be addressed and resolved in the face of this powerful technology.

Stem cells derived from haemopoietic organs

Bone marrow harbours cells that can become parenchymal cells after entering the liver, intestine, skin, lung, skeletal muscle, heart muscle, and central nervous system,[16] in rodent models and in human recipients of marrow or organ transplantation.[17,18] In rodents, haemopoietic organs harbour cells that can also differentiate into functional pancreatic endocrine cells.[19–24]

1–2 months after bone-marrow transplantation, donor-derived cells are found in pancreatic islets of recipient mice.[19] These cells express insulin and genetic markers of β cells. In culture, the cells secrete insulin in response to glucose, and show intracellular calcium fluctuations similar to normal β cells. However, only about 1–3% of the islet cells originate from the transplanted marrow (figure, A).[19] A marrow-derived cell-type with pluripotential capacity to transdifferentiate into various phenotypes has been described.[25] This or a similar cell type might be able to differentiate into pancreatic β cells.

Similar experiments have been done in overtly diabetic mice whose β cells have been destroyed by streptozotocin. After bone-marrow transplantation, blood glucose and insulin concentrations were normal, and survival was better.[20] In islets, marrow-derived cells had differentiated into endothelial cells and occasionally into insulin-expressing cells.[20] Endothelial engraftment was speculated to stimulate the proliferation of local pancreatic progenitors, leading in turn to the increased insulin-producing cell-mass (figure, B).

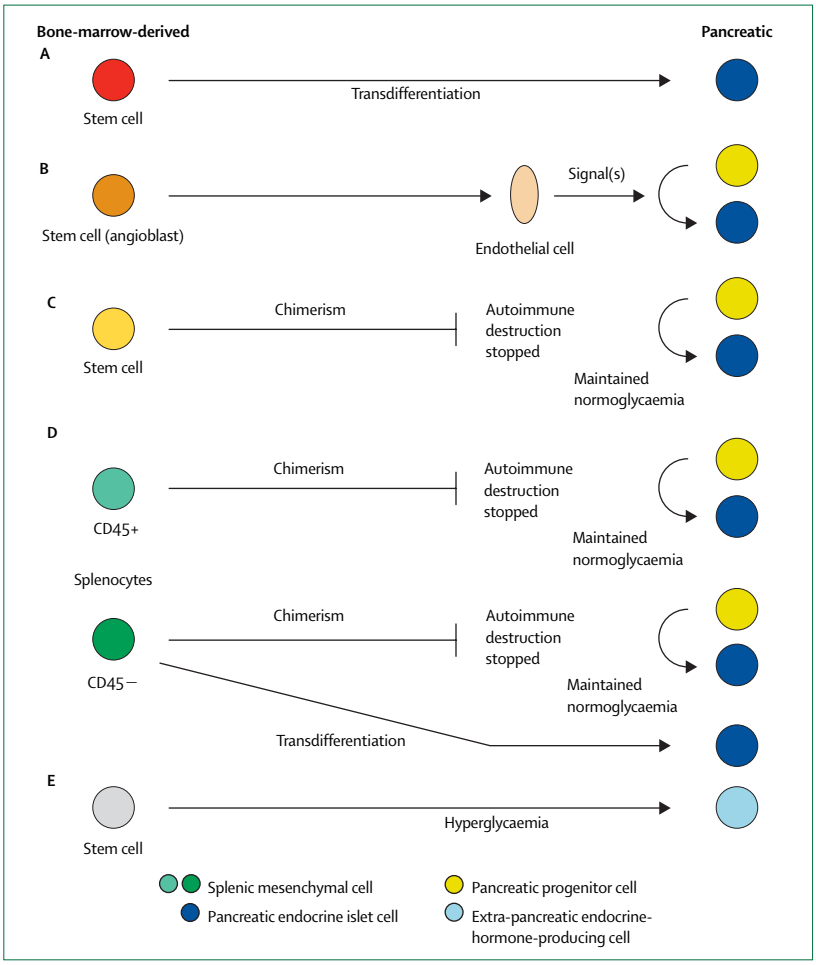

Figure: **Pancreatic islet-cell regeneration after transplantation of cells derived from haemopoietic organs**
A=direct differentiation (transdifferentiation) of bone-marrow-derived stem cells into pancreatic endocrine cells.[19] B=bone marrow contributes to generation of endothelial cells in damaged islets; newly formed endothelial cells stimulate differentiation of locally present host-pancreatic progenitor cells.[20] C=chimerism achieved by bone-marrow transplantation in NOD mice induces immunological tolerance towards β cells.[21] D=spleen mesenchymal-cell transplantation induces immunological tolerance towards β cells.[22,23] E=bone-marrow-derived cells produce pancreatic endocrine hormones at various extrapancreatic sites in hyperglycaemic animals. Adapted from reference 24 with permission.

Although these studies show the possibility of bone-marrow transplantation as a therapeutic approach for β-cell replacement, the immunological destruction of newly regenerated β cells in type 1 diabetes remains a problem. Bone-marrow transplantation induces micro-chimerism. In non-obese diabetic (NOD) mice, an autoimmune model of type 1 diabetes, transplanted with marrow before development of autoimmune diabetes, chimerism prevents diabetes mellitus, presumably by mechanisms involving donor immunoregulatory cells that prevent the host cells from becoming autoreactive against β cells. By contrast, in NOD mice that are already diabetic, induction of chimerism by sublethal or lethal irradiation followed by marrow transplantation does not result in recovery from diabetes.[21] However, when these diabetic transplant-recipients are kept normoglycaemic by insulin therapy, they ultimately recover from diabetes. Pancreatic tissue showed increased proliferative activity and regeneration of β cells. Thus marrow transplantation to induce immunological control plus maintenance of normoglycaemia allowed local β or progenitor cells to proliferate as an adaptive response (figure, C).[21]

Transplantation of mesenchymal cells from the spleen combined with complete Freund's adjuvant led to reversal of diabetes accompanied by regeneration of insulin-producing islets.[22] The transplanted splenic mesenchymal cells differentiate into β cells.[23] Thus splenic mesenchymal cells transplanted under certain conditions seem not only to keep immune destruction of islets in check, but also can transdifferentiate into pancreatic β cells. The relative functions seemed to arise from different sub-populations (figure, D).[23]

Bone-marrow cells can differentiate in vitro under controlled conditions into insulin-expressing cells.[25,26] Such cells, transplanted under the kidney capsule of diabetic rodents, correct glucose. Removal of the grafted kidney returned the animals to a diabetic state.[27]

Cell fusion has been suggested as a mechanism of apparent adaption of bone-marrow-derived cells into an extramedullary phenotype.[24,28] Studies involving pancreatic endocrine-cell differentiation from haemopoietic-organ derivatives largely rule out cell-fusion events as a mechanism of transdifferentiation.[19–23] Bone-marrow-derived extramedullary parenchymal tissue is not always found.[28] Some groups find little if any transdifferentiation[29,30] or bone-marrow derivation of intra-islet endothelial cells in diabetic mice.[31] The last finding was made recently by Andreas Lechner and colleagues. One group reports the generation of insulin-producing cells in liver, adipose tissue, spleen, and bone marrow in rodent models of diabetes mellitus.[32] Bone-marrow transplantation shows that most if not all extrapancreatic insulin-producing cells derive from donor bone-marrow (figure, E).[32]

Stem cells in liver and pancreas

In isolated pancreatic tissue, pancreas-resident progenitor cells might give rise to endocrine islet cells. Human and rodent pancreatic-duct cells,[33,34] islet-derived cells,[35,36] and exocrine tissue[37] contain cells that can differentiate towards a pancreatic endocrine phenotype. These tissues, cultured and differentiated in vitro, have been transplanted and can reverse diabetes mellitus in rodents.

Rodent-liver stem cells and human fetal-liver cells have been differentiated in vitro into insulin-secreting cells by culture methods and/or introduction of β-cell-specific genes. When transplanted, these cells reverse diabetes mellitus in rodents.[39] Cells within liver that can differentiate into insulin-secreting cells after introduction of ß-cell-specific genes have also been seen in vivo after adenoviral gene-delivery into rodents that have been rescued from diabetes for long periods.[40,41]

A bone-fide pancreatic stem cell for β-cell regeneration remains elusive. A genetic-marking study in mice casts doubt on the existence of any β-cell progenitor cells and

suggests that β cells regenerate only by proliferation of existing β cells.[42]

In human beings, early immunological intervention to stop β-cell destruction during the development of type 1 diabetes mellitus allows recovery of pancreatic endocrine function.[43,44] This finding might in part be attributable to recovery in β-cell mass by recruitment of local pancreatic or extrapancreatic progenitor cells that differentiate into β cells and/or proliferation of remaining β cells during protection from immune-mediated destruction.

Acknowledgments

We are partly supported by the Juvenile Diabetes Research Foundation, the National Institutes of Health, and the Singer-Hellman Foundation. We have no conflict of interest to declare.

References

1 Lumelsky N, Blondel O, Laeng P, Vlesco I, Ravion R, McKay R. Differentiaion of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* 2001; **292:** 1398–94.

2 Hori Y, Rulifson IC, Tsai BC, Heit JJ, Cahoy JD, Kim SK. Growth inhibitors promote differentiation of insulin-producing tissue from embryonic stem cells. *Proc Natl Acad Sci USA* 2002; **99:** 16105–10.

3 Kania G, Blyszczuk P, Czyz J, Navarrete-Santos A, Wobus AM. Differentiation of mouse embryonic stem cells into pancreatic and hepatic cells. *Meth Enzymol* 2003; **365:** 287–303.

4 Kahan BW, Jacobson LM, Hullett DA, et al. Pancreatic precursors and differentiated islet cell types from murine embryonic stem cells. *Diabetes* 2003; **52:** 2016–24.

5 Stoffel M, Vallier L, Pedersen RA. Navigating the pathway from embryonic stem cells to beta cells. *Semin Cell Dev Biol* 2004; **15:** 327–36.

6 Blyszczuk P, Czyz J, Kania G, et al. Expression of Pax4 in embryonic stem cells promotes differentiation of nestin-positive progenitor and insulin-producing cells. *Proc Natl Acad Sci USA* 2003; **100:** 998–1003.

7 Miyazaki S, Yamato E, Miyazaki J. Regulated expression of pdx-1 promotes in vitro differentiation of insulin-producing cells from embryonic stem cells. *Diabetes* 2004; **53:** 1030–37.

8 Rajagopal J, Anderson WJ, Kume S, Martinez OI, Melton DA. Insulin staining of ES cell progeny from insulin uptake. *Science* 2003; **299:** 363.

9 Kim D, Gu Y, Ishii M, et al. In vivo functioning and transplantable mature pancreatic islet-like cell clusters differentiated from embryonic stem cell. *Pancreas* 2003; **27:** e34–41.

10 Soria B, Skoudy A, Martin F. From stem cells to beta cells. *Diabetologia* 2001; **44:** 407–15.

11 Assady S, Maor G, Amit M, Itskovitz-Elder J, Skorecki KL, Tzukerman M. Insulin production by human embryonic stem cells. *Diabetes* 2001; **50:** 1691–97.

12 Segev H, Fishman B, Ziskind A, Shulman M, Itskovitz-Eldor J. Differentiation of human embryonic stem cells into insulin-producing clusters. *Stem Cells* 2004; **22:** 265–74.

13 Moritoh Y, Yamato E, Yasui Y, Miyazaki S, Miyazaki J. Analysis of insulin-producing cells during in vitro differentiation from feeder-free embryonic stem cells. *Diabetes* 2003; **52:** 1163–68.

14 Sipione S, Eshpeter A, Lyon JG, Korbutt GS, Bleackley RC. Insulin expressing cells from differentiated embryonic stem cells are not beta cells. *Diabetologia* 2004; **47:** 499–507.

15 Cowan CA, Klimanskaya I, McMahon J, et al. Derivation of embryonic stem-cell lines from human blastocysts. *N Engl J Med* 2004; **350:** 1353–56.

16 Herzog EL, Chai L, Krause DS. Plasticity of marrow-derived stem cells. *Blood* 2003; **102:** 3483–93.

17 Theise ND, Nimmakayalu M, Gardner R, et al. Liver from bone marrow in humans. *Hepatology* 2000; **32:** 11–16.

18 Korbling M, Katz RL, Khanna A, et al. Hepatocytes and epithelial cells of donor origin in recipients of peripheral blood stem cells. *N Engl J Med* 2002; **346:** 738–46.

19 Ianus A, Holz GG, Theise ND, Hussain MA. In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. *J Clin Invest* 2003; **111:** 843–50.

20 Hess D, Li L, Martin M, et al. Bone marrow-derived stem cells initiate pancreatic regeneration. *Nat Biotechnol* 2003; **21:** 763–70.

21 Zorina TD, Subbotin VM, Bertera S, et al. Recovery of endogenous β cells function in the NOD model of autoimmune diabetes. *Stem Cells* 2003; **21:** 377–88.

22 Ryu S, Kodama S, Ryu K, Schoenfeld DA, Faustman DL. Reversal of established autoimmune diabetes by restoration of endogenous beta cell function. *J Clin Invest* 2001; **108:** 63–65.

23 Kodama S, Kühtreiber W, Fujimura S, Dale EA, Faustman DL. Islet regeneration during the reversal of autoimmune diabetes in NOD mice. *Science* 2003; **302:** 1223–26.

24 Kofman A, Theise ND, Hussain MA. Paradigms of adult stem cell therapy for type 1 diabetes mellitus in mice. *Eur J Endocrinol* 2004; **150:** 415–19.

25 Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 2002; **418:** 41–49.

26 Jahr H, Bretzel RG. Insulin-positive cells in vitro generated from rat bone marrow stromal cells. *Transplant Proc* 2003; **35:** 2140–41.

27 Oh SH, Muzzonigro TM, Bae S-H, LaPlante JM, Hatch H, Petersen BE. Adult bone marrow-derived cells trans-differentiating into insulin-producing cells for the treatment of type 1 diabetes. *Lab Invest* 2004; **84:** 607–17.

28 Theise ND, Wilmut I. Cell plasticity: flexible arrangement. *Nature* 2003; **425:** 21.

29 Wagers, AJ, Sherwood RI, Christensen JL, Weissman IL. Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* 2002; **297:** 2256–59.

30 Choi JB, Uchino H, Azuma K, et al. Little evidence of transdifferentiation of bone marrow-derived cells into pancreatic beta cells. *Diabetologia* 2003; **46:** 1366–74.

31 Lechner A, Yang Y-G, Blacken RA, Wang L, Nolan AL, Habener JF. No evidence for significant transdifferentiation of bone marrow into pancreatic β-cells in vivo. *Diabetes* 2004; **53:** 616–23.

32 Kojima H, Fujimiya M, Matsumura K, Nakahara T, Hara M, Chan L. Extrapancreatic insulin-producing cells in multiple organs in diabetes. *Proc Natl Acad Sci USA* 2004; **101:** 2458–63.

33 Bonner-Weir S, Taneja M, Weir GC, et al. In vitro cultivation of human islets from expanded ductal tissue. *Proc Natl Acad Sci USA* 2000; **97:** 7999–8004.

34 Ramiya VK, Maraist M, Arfors KE, Schatz DA, Peck AB, Cornelius JG. Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells. *Nat Med* 2000; **6:** 278–82.

35 Teitelman, G. Induction of beta-cell neogenesis by islet injury. *Diab Metab Rev* 1996; **12:** 91–102.

36 Zulewski H, Abraham EJ, Gerlach MJ, et al. Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. *Diabetes* 2001; **50:** 521–33.

37 Lipsett M, Finegood D. Beta cells neogenesis during prolonged hyperglycemia in rats. *Diabetes* 2002; **51:** 1834–41.

38 Yang L, Li S, Hatch H, et al. In vitro trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. *Proc Natl Acad Sci USA* 2002; **99:** 8078–83.

39 Zalzman M, Gupta S, Giri RK, et al. Reversal of hyperglycemia in mice by using human expandable insulin-producing cells differentiated from fetal liver progenitor cells. *Proc Natl Acad Sci USA* 2003; **100:** 7253–58.

40 Meivar-Levy I, Ferber S. New organs from our own tissues: liver-to-pancreas transdifferentiation. *Trends Endocrinol Metab* 2003; **14:** 460–66.

41 Kojima H, Fujimiya M, Matsumura K, et al. NeuroD-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. *Nat Med* 2003; **9:** 596–603.

42 Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* 2004; **429:** 41–46.

43 Herold KC, Hagopian W, Auger JA, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med* 2002; **346:** 1692–98.

44 Glandt M, Hagopian W, Herold KC. Treatment of type 1 diabetes with anti-CD3 monoclonal antibody. *Rev Endocr Metab Disord* 2003; **4:** 361–68.