Reviews • POST SCREEN

Cell-based treatments for diabetes

Peter M. Jones[1], Monica L. Courtney[2], Christopher J. Burns[2] and Shanta J. Persaud[1]

[1] Beta Cell Group, Division of Reproduction and Endocrinology, King's College London, London SE1 1UL, UK
[2] Endocrinology Section, Biotherapeutics, National Institute for Biological Standardisation and Control, Potters Bar EN6 3QG, UK

In Type 1 diabetes mellitus the insulin-secreting β-cells in pancreatic islets of Langerhans are selectively destroyed by autoimmune assault. Because diabetes is caused by the loss of a single cell type it is amenable to treatment by cell replacement therapy. Advances in islet transplantation procedures have demonstrated that people with Type 1 diabetes can be cured by human islet transplantation, but the severely limited availability of donor islets has restricted the widespread application of this approach, and driven the search for substitute transplant tissues. Recent experimental studies suggest that three separate sources of tissue show therapeutic potential – xenografts from other species, tissue stem cells and embryonic stem cells. Of these, xenografts are closest to clinical application but there are still major obstacles to be overcome. Insulin-expressing cells have been derived from a number of different stem cell populations but embryonic stem cells offer the major advantage of being able, in principle, to provide the vast numbers of cells required for transplantation therapy.

Type 1 diabetes mellitus (T1DM) is a single-cell disorder in which insulin-secreting β-cells in pancreatic islets of Langerhans are irreversibly destroyed by an autoimmune assault, resulting in potentially fatal metabolic dysfunction as a consequence of insufficient circulating levels of insulin. Since the isolation of insulin in 1920s the main therapeutic approach to T1DM has been insulin replacement, but excursions in blood glucose associated with intermittent insulin delivery led to a high risk of long-term complications, causing increased morbidity and mortality. Recent clinical trials, however, have demonstrated that transplantation of human islets of Langerhans can offer a cure for T1DM [1,2]. There are extant problems with current transplantation protocols [2], but the success of islet transplants is proof-of-concept that cell-based treatments for T1D can be effective. This review will focus on one major impediment to the widespread uptake of transplantation therapy for T1DM, which is the very limited availability of suitable transplant material.

An adult human pancreas contains approximately 10^6 islets ($\sim 2 \times 10^9$ β-cells), but these comprise only a minor part of total pancreatic tissue (2–3%) so islets for transplantation must be isolated from whole pancreas by enzymatic digestion, which is an inefficient process. The clinical symptoms of diabetes do not usually become apparent until 60–80% of the β-cell mass is lost, suggesting that glycaemic control can be maintained on 20–40% of normal β-cell mass. Current evidence suggests that a significant fraction of transplanted islets are lost in the immediate post-transplantation period so, to ensure a successful outcome, islet transplantation protocols replace up to 10^6 primary human islets per recipient. A single transplantation may therefore require islets from up to four donor pancreases. At present, the only suitable source of human islets for clinical use is from pancreases of heart-beating, brain-dead donors. This type of organ donor is rare, so current protocols for human islet transplantation are unlikely to make a widespread therapeutic impact on T1DM, with $\sim 10^5$ potential recipients in the UK and $\sim 10^6$ in the USA. This clinical need is therefore driving research into alternative sources of functionally competent, insulin-secreting β-cells as substitutes for donor islets in transplantation therapy.

A number of different cells/tissues have been proposed as potential starting material from which to generate transplant

Corresponding author: Jones, P.M. (peter.jones@kcl.ac.uk)

BOX 1

### Potential sources of β-cells for transplantation therapy
A number of potential sources of material for cell-based therapy of diabetes have been investigated over the past decade. This lists some of the more promising candidates.
- insulin-secreting cell lines
- engineered non-β-cells/gene therapy
- islets/β-cells from other species
- tissue stem cells
  - pancreas
  - bone marrow
  - liver
  - neural
- embryonic stem cells

material, and some of these are shown in Box 1. The physiological properties required of substitute β-cells have been considered in detail elsewhere [3,4] but it is worth considering briefly two essential attributes of such cells. First, they must be able to synthesise and store insulin, and to release it in sufficient amounts to maintain plasma glucose in a narrow range (5–8 mM) in the face of sporadic food intake. Too much insulin is as potentially lethal as too little insulin, so the cells must be able to respond rapidly to changes in plasma glucose in either direction. Pancreatic β-cells have evolved complex stimulus–response coupling mechanisms to monitor and respond to changes in nutrients, hormones and

neurotransmitters (Figure 1), and substitute β-cells will require similar mechanisms to enable a tightly regulated release of insulin in response to environmental cues. Second, the proliferative capacity of the replacement cells must be tightly regulated to avoid post-transplantation expansion of β-cell mass leading to the development of hyperinsulinaemic hypoglycaemia. This is not a problem when using authentic β-cells/islets derived from human donors because they have an exceedingly low proliferative capacity [5], but it is a potential drawback when using cells generated *in vitro* from proliferative precursor populations.

This combination of highly specialised secretory function and controlled proliferative capacity is a challenging target, and some of the suggested starting materials (Box 1) fail to meet one or other of these criteria and so will not be considered in detail in this review. For example, transformed cells derived from human β-cells [6] offer the potential of generating *in vitro* the (thousands of) billions of cells required for transplantation therapy, and insulin-secreting cell lines can be engineered to produce regulated secretory responses [7]. Such cells show unregulated proliferation and form insulinomas *in vivo*, and so are unlikely to offer any therapeutic benefit in the foreseeable future. Another suggested source of replacement β-cells is to engineer non-β-cells to make insulin, and this has been done in a variety of cell types including fibroblasts, skeletal muscle, neuroendocrine, kidney and ovarian cells (for example, see Ref. [8]). This approach has the potential advantage of using the patient's own tissue as a starting material (e.g.
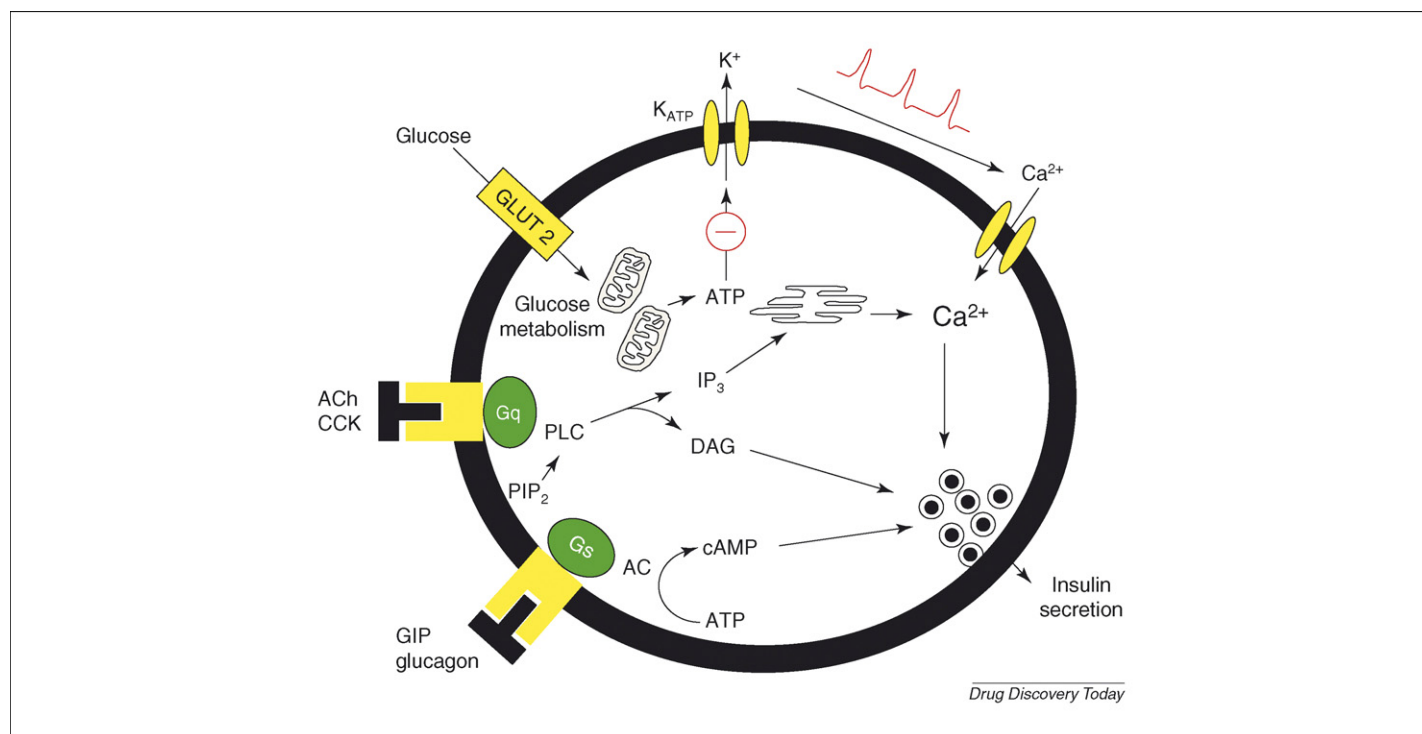
FIGURE 1

Signal transduction in β-cells. β-cells have complex mechanisms to enable them to respond to multiple external cues (nutrient and non-nutrient) and secrete the appropriate amount of insulin to maintain blood glucose within a narrow range (5–8 mM). Briefly, glucose is transported into β-cells on the high capacity GLUT2 transporter and metabolised within the cell, generating ATP which binds to ATP-sensitive K^+-channels (K_{ATP}), leading to closure of the channels. The consequent inhibition of K^+ efflux leads to β-cell plasma membrane depolarisation and opening of voltage-operated Ca^{2+} channels. Extracellular Ca^{2+} enters β-cells resulting in elevations in intracellular Ca^{2+}, activation of Ca^{2+}-sensitive downstream signalling pathways and initiation of insulin secretion. Insulin secretion is also stimulated by ligands such as acetylcholine (ACh) and cholecystokinin (CCK) acting at cell surface receptors linked to IP_3 and DAG generation and those such as glucose-dependent insulinotropic peptide (GIP) and glucagon, which elevate intracellular cyclic AMP.

fibroblasts), but these engineered cells lack the stimulus recognition and response elements that are expressed by authentic β-cells (Figure 1). Their insulin secretory responses are therefore unregulated and are not responsive to physiologically relevant stimuli such as post-prandial changes in blood glucose. There may be some therapeutic benefit to people with diabetes in having a low, maintained basal delivery of insulin via genetically engineered non-β-cells [9], but this would at best be an adjunct to, rather than a replacement for, conventional insulin therapy. The remainder of this review will therefore focus on areas that we consider most likely to generate the quantities of cells required for transplantation while maintaining differentiated function – islets from non human species and the *de novo* generation of functional β-cells from stem cell populations.

Islets/β-cells from other species: xenografts to treat Type 1 diabetes

Using islets of Langerhans from other species is an obvious way of providing the large amounts of functional tissue required for transplantation therapy of diabetes. Most effort in this area has been directed towards using pig islets [10] because: (i) Western countries have pre-existing facilities for high-throughput breeding, rearing and slaughtering of pigs (porcine pancreas as a by-product of pork production was a source of insulin for treating T1DM for many years before recombinant human insulin became widely available); (ii) high islet yields can be obtained from porcine pancreas using techniques similar to those for human islet isolation; and (iii) pigs are amenable to genetic modification to make human insulin or to protect against immune assault. Two important impediments have however restricted the widespread use of pig islets in humans. First, the hyperimmune response to xenografts has proved difficult to avoid. Numerous attempts to hide the transplanted xenografts from the host immune system by islet encapsulation have largely failed to maintain islet viability and insulin secretory responses over the prolonged periods required of transplanted islets. Second, the demonstration that porcine endogenous retroviral (PERV) sequences in the porcine DNA may become activated on xenotransplantation [11] raised the possibility of novel viral infections in humans receiving porcine islet implants. Until recently, these drawbacks made it unlikely that xenotransplantation would find any major clinical application in the treatment of T1DM, but several recent developments have placed it back in the spotlight. In a proof-of-concept study, T-cell-targeted immunosuppressive therapy enabled the use of unmodified pig islets to reverse experimental diabetes in non-human primates for prolonged periods [12]. This work convinced the US-based research charity Diabetes Research and Wellness Foundation to invest a substantial amount of funding in the Spring Point Project (http://www.diabetesinstitute.org/), whose mission is 'to expedite the widespread availability of islet tissue for diabetes care by developing premier source pigs for islet xenotransplantation.' (www.springpointproject.org). Recent work has also suggested that the potential threat of PERVs to xenotransplant recipients may have been over-estimated, because prolonged immunosuppression and exposure to porcine islet xenografts caused no detectable transmission of PERVs in *in vitro* or *in vivo* studies [13].

The renewed interest in porcine islet xenotransplantation has generated some controversial human clinical trials. An Australian company, Living Cell Technologies Ltd., has reported a trial in Moscow in which encapsulated porcine islet cells (DiabeCell) were transplanted into two patients with diabetes, leading to reduced insulin requirements (http://www.lctglobal.com/news/180.php). These workers subsequently reported evidence of residual, viable, encapsulated pig β-cells being retrieved from a transplant recipient almost a decade after xenotransplantation [14]. The approach taken by Living Cell Technologies has however been strongly criticised by the International Xenotransplantation Association as being premature and potentially risky [15]. Similarly, earlier clinical trials of porcine islet transplantation in Mexico [16] have been the subject of extensive criticism about the preclinical data used in justification of the trials, and about the reported efficacy of the trials [17]. The commercial and clinical potential of pig islets as a source of transplant material for treating T1DM seems likely to drive larger, more rigorous and more carefully controlled clinical trials, and the outcome of those trials will determine whether pig islets are a realistic functional substitute for human islets. The concept of islet xenografts has been around for many years without making much progress, but recent events suggest that this is an area worth watching.

Stem cells as a source of substitute β-cells

The recent explosion of interest in the therapeutic potential of stem cells for single cell disorders such as T1DM has generated much research activity in this area. We will focus on recent advances in the generation of functional β-cells from stem cell populations, using a broad classification of stem cells as either tissue stem cells, which are multipotent progenitor cells found in fetal and adult tissues, or as embryonic stem cells, which are pluripotent, undifferentiated cells generated from the inner cell mass of a developing blastocyst and which have the potential to differentiate into any tissue in the embryo.

Tissue stem cells

Tissue stem cells are usually considered to be lineage-restricted, and to mature into the differentiated cells of the host tissue/organ, but evidence is emerging of trans-differentiation into other types of tissue, suggesting that disparate tissues may offer sources of progenitor cells that have the potential to become insulin-expressing cells. Earlier, promising studies using tissue stem cells have so far failed to translate into reliable protocols for generating large numbers of functional β-cells *in vitro*. These studies have been reviewed extensively elsewhere [18–20] so we will summarise recent advances in some promising candidate tissues.

Pancreatic stem cells

The β-cell mass increases during development, and changes with (patho)physiological circumstances such as pregnancy and obesity. The origin(s) of these new β-cells is important when considering how to expand the β-cell mass *in* or *ex vivo*. Recent lineage tracing studies in mice suggest that, under most circumstances including acute pancreatic regeneration, new β-cells arise by a slow but uniform self-renewal of existing β-cells [21,22]. A recent study of β-cell proliferation suggested that, unlike rodent β-cells, adult human β-cells are largely non-proliferative *in vitro* [5], although this has been questioned by another recent study that suggested the de-differentiation of human β-cells into proliferative

'progenitor' cells *in vitro* [23]. On balance, it seems unlikely at present that strategies directed at expanding human β-cell numbers by *in vitro* manipulation will generate substantial amounts of transplant material. In contrast to the lineage tracing studies, numerous reports suggest the existence in adult pancreas of progenitor cells with the potential to differentiate into an insulin-expressing phenotype (e.g. [18–20]). In a recent important study, experimentally induced damage to the mouse pancreas resulted in the upregulation of progenitor cells with the capacity to differentiate into different islet endocrine cells, indicative of the existence of multipotent pancreatic progenitor cells [24]. Putative progenitor cells have been localised previously to the exocrine pancreas (e.g. [20,25]), to pancreatic ducts (e.g. [26,27]) and to endocrine islets (e.g. [28,29]), suggesting a widespread distribution throughout the pancreas. The prospect of isolating, expanding and differentiating adult pancreatic progenitor cells to a β-cell phenotype has obvious therapeutic potential but at present the precise identity of these cells remains elusive, few studies have employed lineage-tracing techniques to demonstrate progenitor to β-cell progression, and the *ex vivo* proliferative capacity of progenitor populations isolated from human pancreas remains to be demonstrated.

Bone marrow-derived stem cells
Bone marrow (BM) contains hematopoietic stem cells and mesenchymal stem cells, both of which exhibit considerable developmental plasticity. An initial report that BM stem cells could engraft into pancreatic islets *in vivo* and differentiate to an insulin-expressing phenotype [30] could not be confirmed by other groups (see Ref. [20]). More recent reports suggest that BM stem cells reversed experimental diabetes *in vivo* by enhancing the regeneration and survival of endogenous β-cells rather than re-populating the islets with trans-differentiated β-cells [31,32]. Several *in vitro* studies have demonstrated that rodent BM stem cells can adopt an insulin-expressing phenotype (e.g. [33]), and driving the phenotype of human BM stem cells by the forced expression of β-cell transcription factors generated cells capable of glucose-responsive insulin secretion [34]. Hematopoietic stem cells isolated from umbilical cord blood differentiated *in vitro* into insulin-expressing cells [35], although no information was provided about the secretory characteristics of these cells. The therapeutic potential of BM stem cells for T1DM is therefore tantalising: experimental studies suggest the potential for generating insulin-expressing/secreting cells, and these cells are particularly attractive for clinical use because they are already routinely collected from adults, purified and used therapeutically, and they also offer the potential for autograft therapy of T1DM.

Liver progenitor cells
Early studies demonstrating that the experimental overexpression of pancreatic transcription factors in hepatic cells *in vivo* produced insulin-expressing cells [36] generated a great deal of interest in the potential of liver cells as a source of replacement β-cells. Liver is relatively accessible for biopsy and has the capacity to regenerate and so is a clinically attractive option for autologous grafting of liver-derived cells in patients with T1DM. A recent study using lentiviral delivery of an insulin gene construct to rat liver *in vivo* demonstrated improved control of blood glucose without trans-differentiation of the liver cells [37], and a number of *in vivo* and *in vitro* studies have demonstrated that liver cells can be induced to express markers and adopt some functional aspects of β-cells (e.g. [36,38–40]). These results are encouraging and some of the genetically modified liver cells express significant amounts of insulin (see Ref. [20]), but there is little evidence to date to suggest that they are amenable to the *in vitro* expansion that is required to generate the amounts of tissue needed for effective transplantation therapy.

Neural stem cells
The use of neural stem cells as β-cell precursors has not been much explored. Subpopulations of neurons express functional elements that are characteristic of β-cells including ATP-dependent K$^+$ channel SUR1 and Kir6.2 subunits, voltage-operated Ca^{2+} channels, GLUT2 glucose transporters and the pancreatic form of glucokinase [41], and the developmental pathways of β-cells and neurons show similarities with many transcription factors (e.g. PDX-1, nkx2.2, nkx6.1, neurogenin-3, NeuroD1/Beta2, Pax4, Pax6 and Isl1) regulating both processes [42,43]. In separate studies fetal rat primary neural stem cells [42] or transformed human neural progenitor cell lines [43] were differentiated *in vitro* towards an insulin-expressing phenotype. Neural stem cells are capable of significant expansion *in vitro* and the biology of human neural stem cells is becoming increasingly well understood [44], so they may offer a future source of β-cell substitutes.

Other tissue stem cells
There have been sporadic reports that progenitor/stem cells from other tissues can be induced to differentiate into insulin-expressing cells, including cells localised to intestinal epithelium, dermis, spleen, salivary gland and blood monocytes. These studies have not always proved to be reproducible, and have been reviewed elsewhere [18–20].

Embryonic stem cells
Embryonic stem (ES) cells have great potential in cell/organ replacement therapies because of two intrinsic properties. First, ES cells can produce cells of all three embryonic germ layers (*pluripotent*). Second, ES cells can proliferate indefinitely *in vitro* if they are maintained in their initial undifferentiated state, so they are capable of producing the large numbers of cells required for transplantation therapies. Initial attempts to differentiate ES cells to insulin-expressing cells used mouse ES (mES) cells [45,46], as did many subsequent studies, because mES cells are much easier to obtain and use than human ES (hES) cells, and because they are not subject to the ethical and legal constraints that accompany hES cells. These initial reports stimulated many studies around the world, and these have been the subject of numerous reviews (e.g. [3,4,47–49]). Although cells destined for transplantation therapy for T1DM would ideally be of human origin there have been relatively few reports of generating insulin-expressing cells from hES cells, so we will focus on some exciting recent advances in this area.

Initial studies demonstrated that hES cells spontaneously differentiate to generate insulin-expressing cells, albeit at a very low frequency, with only 1–3% of cells expressing insulin [50]. Applying selective culture conditions that were initially devised

Reviews • POST SCREEN

for mES cells [45] did not improve the efficiency of β-cell differentiation [51,52], but transplantation into mice along with mouse embryonic dorsal pancreas improved the efficiency of differentiation, suggesting the existence of important *in vivo* cues that were missing from the *in vitro* environment [53]. Over the past decade numerous studies, often in gene knockout mice, have produced a detailed knowledge of the sequence of development of the endocrine pancreas (see Refs. [48,49]). More recent studies using hES cells have used this information to devise differentiation protocols aimed at recapitulating *in vitro* the important *in vivo* signals that drive pluripotent cells first towards definitive endoderm, then to form posterior foregut cells, then to pancreatic endoderm, then to an endocrine progenitor phenotype and finally to a fully differentiated pancreatic endocrine cell (α,β,δ or PP). Culture conditions that encouraged *in vitro* differentiation of hES cells into definitive endoderm [54–56] offered a starting point from which to progress to islet endocrine precursors [55,57,58] and hormone expressing cells [56,58,59]. Cells generated by these *in vitro* protocols showed poor [56] or absent [59] nutrient-induced insulin secretory responses compared to those of primary human β-cells. Transplantation of the pancreatic progenitors derived from hES cells into the *in vivo* environment in mice, however, resulted in enhanced differentiation [57,60], glucose-responsive insulin secretion [60] and the reversal of streptozotocin-induced hyperglycaemia in the host animals [56,57,60]. These studies using similar, but not identical, *in vitro* differentiation protocols offer proof-of-concept that directed differentiation of hES cells based on known developmental signals can generate functional insulin-secreting β-cells. The improved performance of the cells after exposure to the *in vivo* environment in a number of studies [53,57,60] suggests that something is lacking from the current *in vitro* protocols but they offer a useful baseline from which to refine the differentiation process and to determine whether these experimental procedures can be scaled-up to generate the vast numbers of human β-cells that are required for transplantation therapy of T1DM. Progress has been rapid in this area of research in the past 2–3 years, and if it continues at this pace embryonic stem cells may soon fulfil their much-hyped therapeutic potential.

Concluding remarks

Transplantation therapy offers a novel treatment for diabetes and the potential gains, both clinical and commercial, are enormous. The availability of unlimited amounts of functionally competent graft material would allow islet transplantation to evolve from a restricted, experimental treatment to a more widespread applicability, much as has happened for other organ transplantation procedures in the past. The problems associated with graft immunogenicity and autoimmune destruction of engrafted material are common to all sources of replacement β-cells, and beyond the scope of this review, but each of the potential sources of β-cells has specific individual advantages and disadvantages, the balance of which will eventually determine its clinical usefulness. Porcine islets have the great advantages of being fully functional primary islets, and of being available in large numbers, but these are balanced by persistent problems with immune rejection and fears of PERV infection in the recipient. If these problems can be convincingly overcome xenotransplantation may become an effective alternative to human islet transplantation. The concept of autologous grafting of insulin-secreting cells derived from the patient's own tissue stem cells (particularly bone marrow or liver) is very attractive, but the early promise of tissue stem cells in experimental animals has not yet translated into clinically useful material because of problems with restricted proliferative capacity, low levels of insulin expression and poor, or non-existent, insulin secretion. Embryonic stem cells have the required proliferative capacity, and recent studies have demonstrated the differentiation of hES cells to an insulin-secreting phenotype without using clinically unacceptable genetic modification. It remains to be seen whether current experimental protocols can be refined and scaled-up to generate enough cells for transplantation therapy, and whether purification methods of sufficient stringency can be devised to allow the transplantation of the differentiated β-cells while ensuring the absolute exclusion of potentially teratogenic hES cells.

For almost a century the only available treatment for T1DM has been the administration of exogenous insulin to suppress hyperglycaemia. It is now a real possibility that the next decade will see the widespread application of cell-based therapies to cure this disorder.

References

1 Gaglia, J.L. *et al.* (2005) Islet transplantation: progress and challenge. *Arch. Med. Res.* 36, 273–280

2 Marzorati, S. *et al.* (2007) Allogeneic islet transplantation. *Expert. Opin. Biol. Ther.* 7, 1627–1645

3 Burns, C.J. *et al.* (2004) Stem cell therapy for diabetes: do we need to make beta cells? *J. Endocrinol.* 183, 437–443

4 Burns, C.J. *et al.* (2006) Diabetes Mellitus: a potential target for stem cell therapy. *Curr. Stem. Cell Res. Ther.* 1, 255–266

5 Parnaud, G. *et al.* (2008) Proliferation of sorted human and rat beta cells. *Diabetologia* 51, 91–100

6 Narushima, M. *et al.* (2005) A human beta-cell line for transplantation therapy to control type 1 diabetes. *Nat. Biotechnol.* 23, 1274–1282

7 Newgard, C.B. *et al.* (1997) Engineered cell lines for insulin replacement in diabetes: current status and future prospects. *Diabetologia* 40, S42–S47

8 Shaw, J.A. *et al.* (2002) Secretion of bioactive human insulin following plasmid-mediated gene transfer to non-neuroendocrine cell lines, primary cultures and rat skeletal muscle in vivo. *J. Endocrinol.* 172, 653–672

9 Ratanamart, J. and Shaw, J.A. (2006) Plasmid-mediated muscle-targeted gene therapy for circulating therapeutic protein replacement: a tale of the tortoise and the hare? *Curr. Gene Ther.* 6, 93–110

10 Trucco, M. *et al.* (2007) The pig as the donor of pancreatic islets for men. *Vet. Res. Commun.* 31, 27–33

11 van der Laan, L.J. *et al.* (2000) Infection by porcine endogenous retrovirus after islet xenotransplantation in SCID mice. *Nature* 407, 90–94

12 Hering, B.J. *et al.* (2006) Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. *Nat. Med.* 12, 301–303

13 Denner, J. *et al.* (2008) No transmission of porcine endogenous retroviruses (PERVs) in a long-term pig to rat xenotransplantation model and no infection of immunosuppressed rats. *Ann. Transplant.* 13, 20–31

14 Elliott, R.B. *et al.* (2007) Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. *Xenotransplantation* 14, 157–161

15 Grose, S. (2007) Critics slam Russian trial to test pig pancreas for diabetics. *Nat. Med.* 13, 390–391

16 Valdes-Gonzalez, R.A. et al. (2007) Three-yr follow-up of a type 1 diabetes mellitus patient with an islet xenotransplant. Clin. Transplant. 21, 352–357

17 Sykes, M. and Cozzi, E. (2006) Xenotransplantation of pig islets into Mexican children: were the fundamental ethical requirements to proceed with such a study really met? Eur. J. Endocrinol. 154, 921–922

18 Limbert, C. et al. (2008) Beta-cell replacement and regeneration: Strategies of cell-based therapy for type 1 diabetes mellitus. Diabetes Res. Clin. Pract. 79, 389–399

19 Burke, Z.D. et al. (2007) Stem cells in the adult pancreas and liver. Biochem. J. 404, 169–178

20 Efrat, S. (2008) Beta-cell replacement for insulin-dependent diabetes mellitus. Adv. Drug Deliv. Rev. 60, 114–123

21 Dor, Y. et al. (2004) Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature 429, 41–46

22 Teta, M. et al. (2007) Growth and regeneration of adult beta cells does not involve specialized progenitors. Dev. Cell 12, 817–826

23 Russ, H. et al. (2008) In vitro proliferation of cells derived from adult human β-cells revealed by cell-lineage tracing. Diabetes 57, 1575–1583

24 Xu, X. et al. (2008) Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 132, 197–207

25 Zhao, M. et al. (2007) Evidence for the presence of stem cell-like progenitor cells in human adult pancreas. J. Endocrinol. 195, 407–414

26 Bonner-Weir, S. et al. (2000) In vitro cultivation of human islets from expanded ductal tissue. Proc. Natl. Acad. Sci. U.S.A. 97, 7999–8004

27 Yatoh, S. et al. (2007) Differentiation of affinity-purified human pancreatic duct cells to beta-cells. Diabetes 56, 1802–1809

28 Zulewski, H. et al. (2001) Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes. Diabetes 50, 521–533

29 Davani, B. et al. (2007) Human islet-derived precursor cells are mesenchymal stromal cells that differentiate and mature to hormone-expressing cells in vivo. Stem Cells 25, 3215–3222

30 Ianus, A. et al. (2003) In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J. Clin. Invest. 111, 843–850

31 Hasegawa, Y. et al. (2007) Bone marrow (BM) transplantation promotes beta-cell regeneration after acute injury through BM cell mobilization. Endocrinology 148, 2006–2015

32 Urban, V.S. et al. (2008) Mesenchymal stem cells cooperate with bone marrow cells in therapy of diabetes. Stem Cells 26, 244–253

33 Tang, D.Q. et al. (2004) In vivo and in vitro characterization of insulin-producing cells obtained from murine bone marrow. Diabetes 53, 1721–1732

34 Karnieli, O. et al. (2007) Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. Stem Cells 25, 2837–2844

35 Sun, B. et al. (2007) Induction of human umbilical cord blood-derived stem cells with embryonic stem cell phenotypes into insulin producing islet-like structure. Biochem. Biophys. Res. Commun. 354, 919–923

36 Ferber, S. et al. (2000) Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. Nat. Med. 6, 568–572

37 Elsner, M. et al. (2008) Diabetes therapy by lentiviral hepatic insulin gene expression without transformation of the liver. Diabetologia 51, 694–695

38 Kojima, H. et al. (2003) Neuro D-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. Nat. Med. 9, 596–603

39 Li, W.C. et al. (2005) In vitro transdifferentiation of hepatoma cells into functional pancreatic cells. Mech. Dev. 122, 835–847

40 Yatoh, S. et al. (2007) NeuroD and reaggregation induce beta-cell specific gene expression in cultured hepatocytes. Diabetes Metab. Res. Rev. 23, 239–249

41 Yang, X.J. et al. (1999) Hypothalamic glucose sensor: similarities to and differences from pancreatic beta-cell mechanisms. Diabetes 48, 1763–1772

42 Burns, C.J. et al. (2005) The in vitro differentiation of rat neural stem cells into an insulin-expressing phenotype. Biochem. Biophys. Res. Commun. 326, 570–577

43 Hori, Y. et al. (2005) Differentiation of insulin-producing cells from human neural progenitor cells. PLoS. Med. 2, 103

44 Zhao, C. et al. (2008) Mechanisms and functional implications of adult neurogenesis. Cell 132, 645–660

45 Lumelsky, N. et al. (2001) Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science 292, 1389–1394

46 Rajagopal, J. et al. (2003) Insulin staining of ES cell progeny from insulin uptake. Science 299, 363

47 Colman, A. (2004) Making new beta cells from stem cells. Semin. Cell Dev. Biol. 15, 337–345

48 Madsen, O.D. (2005) Stem cells and diabetes treatment. APMIS 113, 858–875

49 Docherty, K. et al. (2007) Embryonic stem cell therapy for diabetes mellitus. Semin. Cell Dev. Biol. 18, 827–838

50 Assady, S. et al. (2001) Insulin production by human embryonic stem cells. Diabetes 50, 1691–1697

51 Segev, H. et al. (2004) Differentiation of human embryonic stem cells into insulin-producing clusters. Stem Cells 22, 265–274

52 Baharvand, H. et al. (2006) Generation of insulin-secreting cells from human embryonic stem cells. Dev. Growth Diff. 48, 323–332

53 Brolen, G.K. et al. (2005) Signals from the embryonic mouse pancreas induce differentiation of human embryonic stem cells into insulin-producing beta-cell-like cells. Diabetes 54, 2867–2874

54 D'Amour, K.A. et al. (2005) Efficient differentiation of human embryonic stem cells to definitive endoderm. Nat. Biotechnol. 23, 1534–1541

55 Xu, X. et al. (2006) Endoderm and pancreatic islet lineage differentiation from human embryonic stem cells. Cloning Stem Cells 8, 96–107

56 Jiang, W. et al. (2007) In vitro derivation of functional insulin-producing cells from human embryonic stem cells. Cell Res. 17, 333–344

57 Shim, J.H. et al. (2007) Directed differentiation of human embryonic stem cells towards a pancreatic cell fate. Diabetologia 50, 1228–1238

58 Micallef, S.J. et al. (2007) Endocrine cells develop within pancreatic bud-like structures derived from mouse ES cells differentiated in response to BMP4 and retinoic acid. Stem Cell Res. 1, 25–36

59 D'Amour, K.A. et al. (2006) Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat. Biotechnol. 24, 1392–1401

60 Kroon, E. et al. (2008) Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat. Biotechnol. 26, 443–452

Reviews • POST SCREEN