61 Other Future Directions

Stem Cell Therapy in Diabetes

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Islet Transplantation

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Gene Therapy

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Stem Cell Therapy in Diabetes

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Keypoints

- Stem cells are self-renewing cells that possess the ability to generate daughter cells that produce large numbers of differentiated progeny. They can be divided into two broad categories: embryonic stem cells and adult stem cells.
- Embryonic stem cells are derived from the inner cell mass of mammalian blastocysts and can give rise to all of the differentiated tissues of the embryo proper, including the β-cells of the pancreas.
- Induced pluripotent stem cells are embryonic stem cell-like cells derived from adult cells such as skin fibroblasts in a process called reprogramming.
- Human embryonic stem cells and induced pluripotent stem cells have been shown to produce insulin-producing cells by stepwise approach in culture.
- Adult stem cells have been identified in many organs, where they participate in tissue repair and homeostasis. No definitively identified stem cell population has been described in pancreas.
- Lineage-tracing experiments suggest that β-cell mass is maintained primarily by replication of pre-existing β-cells during normal adult life in mice. After injury, stem/progenitor cells may be recruited to produce additional islet cells, but the identity of these pancreatic stem/progenitor cells remains unclear.
- Pancreatic exocrine cells can be directly converted to become β-cells *in vivo* in a process called lineage reprogramming.

Textbook of Diabetes, 4th edition. Edited by R. Holt, C. Cockram, A. Flyvbjerg and B. Goldstein.

Introduction

The aim of stem cell therapy in the treatment of type 1 diabetes mellitus (T1DM) is to provide a source of cells that are identical or nearly identical to β-cells. Stem cells by definition are self-renewing, and in some cases they can be propagated clonally from a single cell. These properties allow a degree of reproducibility that is unusual for a cell-based therapeutic vehicle. It may be possible to engineer stem cells to evade immune recognition. It is also now possible to derive patient-specific induced pluripotent cells (iPS) that fully match individual patients immunologically. Encouraging progress has been made in recent years to derive insulin-producing cells from either embryonic stem cells or exocrine cells. Future efforts will be focused on continued improvement of the derivation efficiency and fidelity of β-cells, with the ultimate aim of producing sufficient numbers of transplantable mature β-cells that can rescue hyperglycemic conditions.

General definitions: adult and embryonic stem cells

Stem cells are defined functionally. They are capable of self-renewal and possess the ability to generate daughter cells that produce large numbers of differentiated progeny [1]. They can be divided into two broad categories: embryonic stem cells and adult stem cells. Embryonic stem cells are derived from the inner cell mass of the mammalian blastocyst (Figure 61.1) and are said to be pluripotent because they give rise to all of the fully differentiated tissues of the embryo proper, including the products of all three embryonic germ layers and thus the β-cells of the pancreas (Figure 61.2). Fortunately, conditions have been described that permit the long-term culture of these cells *in vitro* without chromosomal aberration or loss of potency to form diverse tissues (Figure 61.3) [2–6]. Indeed, cell lines can be created by the propagation of a single cell, ensuring homogeneous cell populations that can subsequently be used as starting material for β-cell differentiation experiments.

Adult stem cells are thought to be rare cells and are known to participate in the repair or regeneration of certain tissues, most notably in the bone marrow [7,8]. Furthermore, stem cell populations are involved in the tissue homeostasis of the liver, the brain, the skeletal muscle and the skin [9–13]. Adult stem cells, like their embryonic counterparts, are capable of self-renewal and retain the ability to generate large numbers of differentiated progeny, but they are traditionally thought to produce a more limited number of cell types. Although there is some evidence to suggest the existence of a pancreatic stem cell function, no cell type or marker of such a putative cell has been identified. In fact,

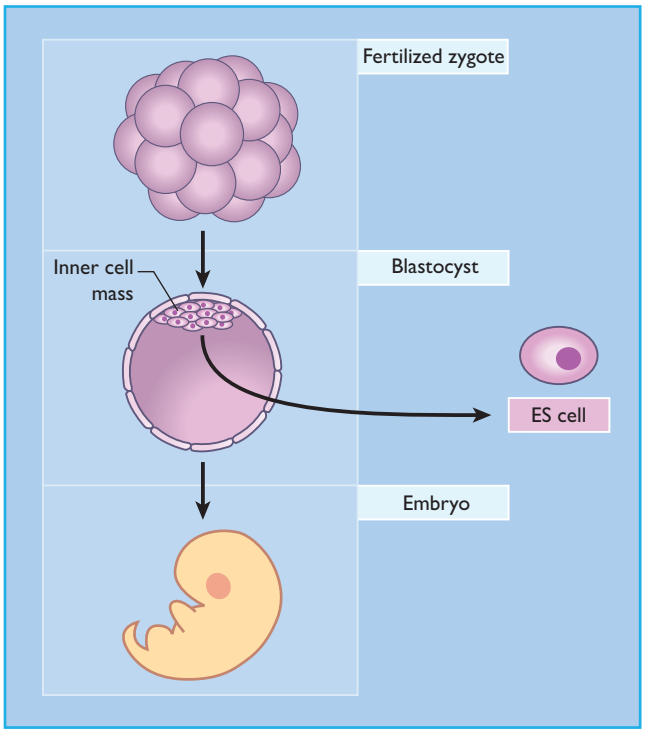

Figure 61.1 Derivation of embryonic stem cells. After fertilization, the single-cell human or mouse zygote undergoes cleavage to produce a multicellular blastocyst. The blastocyst stage of mammalian development occurs well before cytodifferentiation and organogenesis. Cells from the inner cell mass, which normally give rise to the tissues of the embryo proper, can be cultured *in vitro* to produce embryonic stem cells. ES, embryonic stem.

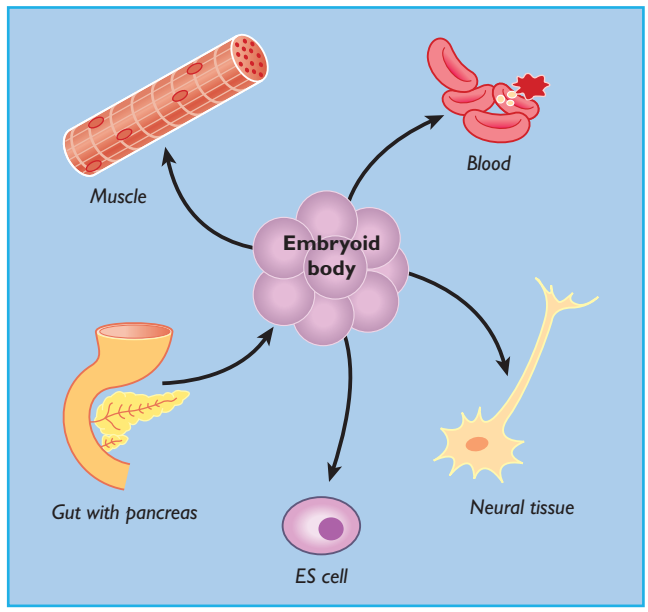

Figure 61.2 The pluripotent nature of embryonic stem (ES) cells. Embryonic stem cells can be cultured *in vitro* indefinitely. When allowed to aggregate in suspension culture, forming embryoid bodies, they differentiate into derivatives of all three embryonic germ layers. This includes ectodermal tissues such as neurons, mesodermal tissues such as muscle cells and blood, and endodermal tissues such as β-cells. Although there are many cell types in embryoid bodies, they are often disorganized and do not adopt the same patterned structure found in mature embryos and adults (e.g. actual muscle, ganglia, gut or islets).

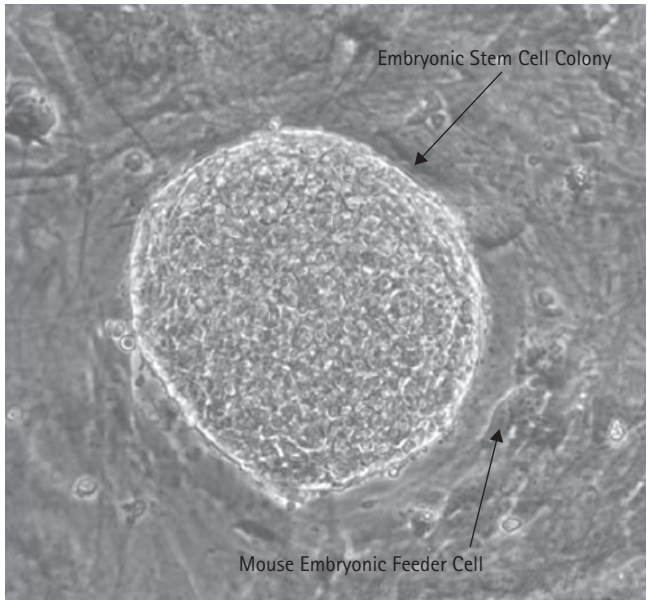

Figure 61.3 A human embryonic stem cell colony is composed of hundreds to thousands of homogeneous cells grown in a Petri dish along with hundreds of other similar stem cell colonies. One colony is shown here. These colonies are grown on mouse embryonic fibroblasts, which provide signals preventing embryonic stem cell differentiation. This allows repeated, near-indefinite subculture of embryonic stem cells. When removed from the murine feeder layer, the stem cells spontaneously differentiate into a wide variety of cell types (not shown).

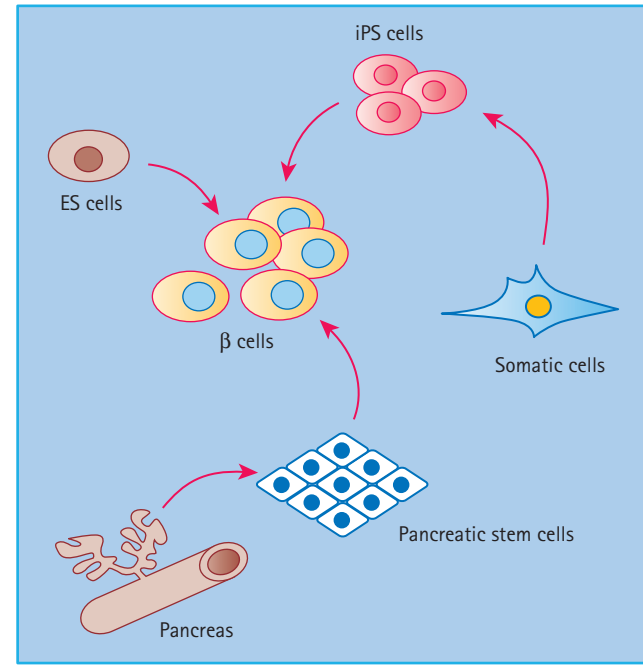

Figure 61.4 Potential stem-cell sources of β-cells. In theory, one can derive pancreatic β-cells from a number of different stem cell sources. Embryonic stem cells from the mammalian blastocyst have the broadest capacity for differentiation. Similarly, induced pluripotent cells (iPS) derived from skin fibroblasts may also give rise to all cell types in the body, including β-cells. Pancreatic stem cells may be the most direct path to β-cells, but their identity is still unclear. ES, embryonic stem.

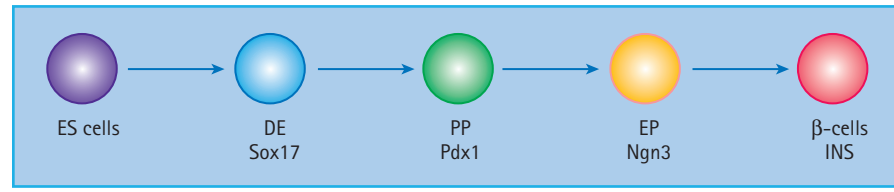

Figure 61.5 Stepwise differentiation from embryonic stem (ES) cells to β-cells. DE, definitive endoderm; PP, pancreatic progenitor; EP, endocrine progenitor; Ngn3, neurogenin 3; INS, insulin.

it is unclear whether the concept of an adult stem cell represents a single well-defined cell type or a common property of a heterogeneous population of different cells [1].

Differentiating β-cells from human embryonic stem cells

Embryonic stem (ES) cells, with a virtually endless replicative capacity and the potential to differentiate into most cell types, provide nearly unlimited starting material to generate differentiated cells for study and clinical therapy [14]. By mimicking signals used during embryonic pancreatic development, to the extent that they are known, a stepwise protocol is being explored to differentiate human ES cells into functional β-cells (Figure 61.4). This involves directing ES cells first to form definitive endoderm, then $Pdx1^+$ pancreatic progenitors, followed by the formation of endocrine progenitors and, finally, $insulin^+$ β-cells (Figure 61.5). Knowledge from pancreatic development provides candidate factors that promote the progression from one step to another, and marker genes that can be used to recognize the cells at each developmental stage.

Using a protocol based on the combination of candidate factors suggested by embryonic pancreatic development, significant advances have been made to produce insulin-producing cells from human ES cells [15–17]. Briefly, human ES cells were first treated with Wnt 3a and Activin A, which can result in approximately 70% of the cells expressing SOX17 [15], a marker of definitive endoderm. In the second step, manipulation of several signaling pathways that include suppression of sonic hedgehog (SHH) and bone morphogenetic protein (BMP), and activation of retinoic acid (RA) [16,17], allow the cells to progress into a

pancreatic and endocrine progenitor stage. This heterogeneous progenitor population can be further differentiated and matured into various pancreatic endocrine cell types including insulin-secreting cells either in Petri dish [16] or after being transplanted into mice [17]. The endocrine cells appear to organize into islet-like structures and gain the ability to ameliorate hyperglycemia.

As an alternative to growth factors as inducers, an unbiased chemical screen approach has been used to identify cell-permeable small molecules to direct human ES cells to pancreatic lineage. In the ideal case, as noted by many others, small molecule inducers would be less expensive, more easily controlled, and possibly more efficient than growth factors in directing differentiation. High content chemical screening has led to the identification of a small molecule, (-)-Indolactam V (ILV), that induces human ES cell differentiation to pancreatic progenitors. These pancreatic progenitors can contribute to insulin-secreting cells both *in vitro* and *in vivo* [18].

Despite recent successes with the human ES cell approach, major challenges remain. The efficiency and consistency of β-cell derivation from human ES cells require improvement. The resulting β-like-cells need to be extensively validated to confirm their function [16] and the possibility of tumor formation after transplantation [17] must be fully addressed. New reagents and methodologies of human ES cell differentiation will need to be developed to overcome these challenges.

Human ES cell lines are derived from blastocyst-stage embryos. Recently, significant progress has been made in reprogramming differentiated human skin fibroblasts to a pluripotent state by introducing defined transcription and other reprogramming factors [19–21]. The resulting pluripotent cells are called induced pluripotent stem cells (iPS cells). iPS cells derived from people with diabetes have recently been shown to differentiate into three germ layers [22]. These DiPS cells could provide an important reagent for modeling diabetes pathology and investigating new therapies.

Evidence for the existence of pancreatic stem cells

In principle, tissue turnover in the adult can occur by the differentiation of adult stem cells (e.g. in skin and intestine), or by the replication of existing differentiated cells. In adult pancreas, there is strong evidence suggesting that new β-cells are generated primarily by the replication of pre-existing β-cells during normal adult homeostasis, as well as during β-cell regeneration induced by either partial removal of the pancreas or specific ablation of β-cells [23–27].

The evidence for the existence of adult pancreatic stem cells is indirect and comes from studies of regenerating pancreata. In these models, a chemical or surgical injury is induced in the adult pancreas. After chemical or surgical pancreatectomy in the islet, a burst of replicative activity in the pancreatic duct cells has also been reported. BrdU label disappears from the ducts and then increases in pancreatic exocrine and endocrine tissue, suggesting that cells may be recruited from ducts to form islets and acini [28]. Groups of cells that appear to be budding from the ducts have been suggested to represent cells recruited from a ductal stem cell compartment. The early burst in epithelial duct proliferation following pancreatectomy is reported to be accompanied by an increase in Pdx1 protein in the replicating duct cells [29]. Pdx1 is a protein that is essential for exocrine and endocrine pancreas formation. In the regenerating hamster pancreas, there are nests of cells near ducts that express insulin and glucagon, suggesting that duct cells may differentiate into hormone-producing cells that subsequently migrate into the islets [30]. In addition, after chemical pancreatectomy with streptozocin (streptozotocin), a cell population that coexpress hormones appears in the islets themselves, including Pdx1–somatostatin cells and somatostatin–insulin cells. These double-positive cells have been noted in the developing pancreas, but their exact role in development of the β-cell lineage is unclear. Nonetheless, it has been suggested that the presence of such cells implies neogenesis from a stem cell compartment located within the islets themselves [31].

It has recently been demonstrated that following partial duct ligation, a progenitor population expressing neurogenin 3 (Ngn3), a marker of embryonic endocrine progenitors, appears in adult mice. These $Ngn3^+$ cells can further give rise to new islet cells, including β-cells after transplantation into cultured embryonic pancreata [32]. This study provides an example of the existence of β-cell progenitors in adult pancreas; however, it is not clear whether this event represents the mobilization of existing adult endocrine progenitor cells that already express low levels of Ngn3, activation of dormant stem-like cells, or conversion of other cells into endocrine cells following injury. In addition, the number of $Ngn3^+$ progenitors detected following injury is very low and the molecules triggering the regeneration event remains unknown.

Taken together, the work on regenerating pancreata suggests the possibility of an existing precursor or stem cell; however, no clearly identified subpopulation of cells that has the capacity for self-renewal and β-cell differentiation has yet been identified as the pancreatic stem cell compartment. If such a pancreatic stem cell compartment does exist, its physiologic significance, in terms of the number of new β-cells produced, needs to be compared with the demonstrable capacity of β-cells for self-duplication.

Lineage reprogramming

In rare cases, adult cells of one lineage may be converted directly into cells of another lineage [33]. These phenomena are broadly defined as lineage reprogramming. For the pancreas, there is some experimental evidence suggesting that non-β-cells, such as liver cells, pancreatic duct cells and exocrine cells, may be converted to β-like-cells in culture [34]. In most of these reported

cases, however, the extent to which the resulting cells resemble true β-cells is often unclear. The molecular mechanism of these reported conversion events also remain largely unknown.

Recently, it has been shown that mature exocrine cells of the pancreas can be reprogrammed to become β-like-cells *in vivo* with a simple combination of three transcription factors. The induced cells closely resemble endogenous islet β-cells in morphology, ultrastructure, molecular signatures and function [35]. There are several issues that need to be resolved before the *in vivo* lineage reprogramming approach can be applied to clinical therapy. For example, the induced β-cells persist as individual cells or small clusters and do not organize into islets. Moreover, viruses currently used to express the reprogramming factors would need to be replaced by safer reagents such as chemical compounds. Furthermore, given the difficulty of biopsying human pancreas, β-cell reprogramming should be accomplished directly *in vivo*, or alternatively, other more easily accessible starting cell populations, such as adult liver cells and skin fibroblasts, may be used to produce β-cells.

Future issues regarding human transplantation of cell-based therapies

There are several promising strategies for the generation of β-cells, from human embryonic stem cells, putative adult stem cells or lineage reprogramming. Nonetheless, a number of issues must be resolved before cells derived by these methods can be used in human transplantation. Immune rejection is prime amongst these concerns. Although the recent success with non-steroid immunosuppression is encouraging, it may be possible to engineer stem cells to be immunologically silent. The development of iPS technology now allows derivation of patient-specific stem cells and immunologically fully matched β-cells. In addition, considerable work also needs to be done to eliminate the risk of neoplasia. We must demonstrate that cells derived from ES cells are not transformed and that they are free from contaminating undifferentiated cells that could go on to produce teratomas in graft recipients. Cells must also be shown to be free of pathogens. Because human ES cells are currently grown on murine feeder cell lines, pathogens associated with xenotransplantation must be considered. Finally, we must assess the stability of the β-cell phenotype in grafts and the longevity of cells once transplanted. Ideally, these transplants would include not only differentiated β-cells, but also pancreatic stem cells with long-term reconstituting activity.

References

1 Blau HM, Brazelton TR, Weimann JM. The evolving concept of a stem cell: entity or function? *Cell* 2001; **105**:829–841.

2 Evans MJ, Kaufman MH. Establishment in culture of pluripotent cells from mouse embryos. *Nature* 1981; **292**:154–156.

3 Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, *et al.* Embryonic stem cell lines derived from human blastocysts. *Science* 1998; **282**:1145–1147.

4 Bongo A, Fong CY, Ng SC, Ratnam S. Isolation and culture of inner cell mass cells from human blastocysts. *Hum Reprod* 1994; **9**:2110–2117.

5 Reubinoff BE, Pera MF, Fong CY, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts. *Nat Biotechnol* 2000; **18**:399–404.

6 Amit M, Carpenter MK, Inokuma MS, Chui CP, Harris CP, Waknitz MA, *et al.* Clonally derived human embryonic stem cells maintain pluripotency and proliferative potential for prolonged periods in culture. *Dev Biol* 2000; **227**:271–278.

7 Morrison SJ, Weissman IL. The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity* 1994; **1**:661–673.

8 Weissman IL. Stem cells as units of development, units of regeneration, and units of evolution. *Cell* 2000; **100**:157–168.

9 Smythe GM, Hodgetts SI, Grounds MD. Immunobiology and future of myoblast transfer therapy. *Mol Ther* 2000; **1**:304–313.

10 Gage FH. Mammalian neural stem cells. *Science* 2000; **287**:1433–1438.

11 Qian X, Shen Q, Goderie SK, He W, Capela A, Davis AA, *et al.* Timing of CNS cell generation: a programmed sequence of neuron and glial cell production from isolated murine cortical stem cells. *Neuron* 2000; **28**:69–80.

12 Thorgeirsson SS, Evarts RP, Bisgaard HC, Fujio K, Hu Z. Hepatic stem cell compartment: activation and lineage commitment. *Proc Soc Exp Biol Med* 1993; **204**:253–260.

13 Taylor G, Lehrer MS, Jensen PJ, Sun TT, Lavker RM. Involvement of follicular stem cells in forming not only the follicle but also the epidermis. *Cell* 2000; **102**:451–461.

14 *Regenerative Medicine*. Department of Health and Human Services, NIH, Medical and Scientific Illustration Washington, DC Terese Winslow, Washington, DC, 2006.

15 D'Amour KA, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE. Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat Biotechnol* 2005; **23**:1534–1541.

16 D'Amour KA, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG, *et al.* Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol* 2006; **24**:1392–1401.

17 Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S, *et al.* Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells *in vivo*. *Nat Biotechnol* 2008; **26**:443–452.

18 Chen S, Borowiak M, Fox JL, Maehr R, Osafune K, Davidow L, *et al.* A small molecule that directs differentiation of human ESCs into the pancreatic lineage. *Nat Chem Biol* 2009; **5**:258–265.

19 Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**:861–872.

20 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**:663–676.

21 Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, *et al.* Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007; **318**:1917–1920.

22 Park IH, Arora N, Huo H, Maherali N, Ahfeldt T, Shimamura A, *et al.* Disease-specific induced pluripotent stem cells. *Cell* 2008; **134**:877–886.
23 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.
24 Brennand K, Huangfu D, Melton D. All beta cells contribute equally to islet growth and maintanance. *PLoS Biol* 2007; **5**:e163.
25 Lee CS, De Leon DD, Kaestner KH, Stoffers DA. Regeneration of pancreatic islets after partial pancreatectomy in mice does not involve the reactivation of neurogenin-3. *Diabetes* 2006; **55**:269–272.
26 Nir T, Melton DA, Dor Y. Recovery from diabetes in mice by beta cell regeneration. *J Clin Invest* 2007; **117**:2553–2561.
27 Teta M, Rankin MM, Long SY, Stein GM, Kushner JA. Growth and regeneration of adult beta cells does not involve specialized progenitors. *Dev Cell* 2007; **12**:817–826.
28 Bonner-Weir S, Baxter LA, Schuppin GT, Smith FE. A second pathway for regeneration of adult exocrine and endocrine pancreas. *Diabetes* 1993; **42**:1715–1720.
29 Sharma A, Zangen DH, Reitz P, Taneja M, Lissauer ME, Miller CP, *et al.* The homeodomain protein idx1 increases after an early burst of proliferation during pancreatic regeneration. *Diabetes* 1999; **48**:507–513.
30 Rosenberg L, Rafaeloff R, Clas D, Kakugawa Y, Pittenger G, Vinik AI, *et al.* Induction of islet cell differentiation and new islet formation on the hamster: further support for a ductular origin. *Pancreas* 1996; **13**:38–46.
31 Fernandes A, King LC, Guz Y, Stein R, Wright CV, Teitelman G. Differentiation of new insulin-producing cells is induced by injury in adult pancreatic islets. *Endocrinology* 1997; **138**:1750–1762.
32 Xu X, D'Hoker J, Stange G, Bonné S, De Leu N, Xiao X, *et al.* Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell* 2008; **132**:197–207.
33 Slack JM. Metaplasia and transdifferentation: from pure biology to the clinic. *Nat Rev Mol Cell Biol* 2007; **8**:369–378.
34 Minami K, Okuno M, Miyawaki K, Okumachi A, Ishizaki K, Oyama K, *et al.* Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. *Proc Natl Acad Sci U S A* 2005; **102**:15116–15121.
35 Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. *In vivo* programming of adult pancreatic exocrine cells to beta-cells. *Nature* 2008; **455**:627–632.

Future Directions: Islet Transplantation

Angela Koh, Peter Senior & A.M. James Shapiro

Keypoints

- Islet transplantation is a promising treatment for patients with type 1 diabetes with severe hypoglycemia, hypoglycemia unawareness and/or glycemic lability.
- Successful islet transplantation can lead to insulin independence, but this is usually not maintained in the long term.
- Graft function is preserved for several years beyond the loss of insulin independence, resulting in better glycemic control in spite of a return to insulin use.
- Current immunosuppression protocols include the use of more potent induction agents which may lead to better long-term outcomes.
- The side effects of maintenance immunosuppression are better understood, and appropriate dose titration and/or change to alternative agents have led to fewer and more tolerable side effects.
- The long-term impact of islet transplantation on diabetes-related complications is not fully elucidated, but there appears to be stabilization of microvascular and macrovascular disease.

Introduction

The discovery of insulin dramatically changed the outcome for patients with type 1 diabetes mellitus (T1DM) and the acute lethal complications of diabetes, such as diabetic ketoacidosis, could be effectively treated. The improved survival, however, allowed the development of the secondary complications of diabetes [1]. Typically, the patient with T1DM develops retinopathy, nephropathy, neuropathy or vascular disease over time. It was not until 1993 that definitive proof for the value of good glycemic control was established. The landmark Diabetes Control and Complications Trial (DCCT) study showed that the microvascular complications of diabetes could be delayed or avoided by good glycemic control, which required intensive insulin therapy [2]. Such therapy was associated with a hemoglobin A_{1c} (HbA_{1c}) that was 1% (11 mmol/mol) lower than that of the control group. It should be noted that the HbA_{1c} was not normalized and in the intensively treated group remained at least 1% (11 mmol/mol) above the upper limit of normal. Similar evidence was provided by the UK Prospective Diabetes Study (UKPDS) in T2DM with microvascular disease, showing a significant improvement for a decline of 1% (11 mmol/mol) in the HbA_{1c} [3]. Thus, glycemic control is essential if the microvascular complications of diabetes are to be prevented.

Controlling glycemia with exogenous insulin remains a challenge. Given the non-physiologic subcutaneous route of insulin administration, its inherent delay in absorption, the variability of serum levels obtained and the systemic versus portal venous delivery, it is perhaps remarkable how well the glucose levels are actually controlled. The newer insulin analogs have removed

some of the variability and delay in absorption, but even with intensive therapy it is difficult to achieve normoglycemia. Continuous subcutaneous insulin administration helps further, but neither earlier nor more recent studies have managed to normalize glucose values [4,5]. Carbohydrate counting and attention to diet improves the quest for normoglycemia but does not offer a complete solution. The largest study using all available tools to optimize therapy was still associated with an increased risk of hypoglycemia [2]. In the DCCT, the risk for severe hypoglycemia (defined as needing third-party assistance) was increased threefold. Concern about hypoglycemia remains the limiting factor in attempts to achieve optimal glycemic control.

Many efforts have been made to generate a closed-loop system for glucose control. The first successful efforts were with the Biostator®, which involved continuous monitoring of blood glucose and then an insulin infusion using a computer-derived algorithm [6]. Although effective for a short period of time, it was impractical being bulky and plagued with flow problems from the double lumen catheter being used for sensing the glucose level. Insulin infusion pumps especially using insulin analogs (such as lispro or insulin aspart) have helped but are not closed-loop systems and require frequent glucose monitoring and rarely result in normal glycemia without severe hypoglycemia. The goal of any closed-loop system is to have reliable glucose sensing linked to appropriate insulin delivery on a continuous basis. Mechanical closed-loop systems may in the future achieve this but have been fraught with problems despite early promise. Transplantation of islets of Langerhans or of the whole pancreas can achieve a physiologic closed-loop system today.

Background history

Transplantation of pancreatic tissue was first tried in 1890 when a surgeon in England transplanted fragments of a sheep's pancreas into a boy with diabetic ketoacidosis [7]. The immune barriers, unknown at the time, were insurmountable, especially for a xenograft. The modern era of transplantation began in the 1960s when the use of steroids, especially when combined with azathioprine, allowed successful renal transplantation. The steroids block several cytokines and act as inhibitors of antigen presentation, and azathioprine is an inhibitor of purine synthesis and thus impedes the generation of active T cells. Cyclosporine A (CsA), a calcineurin inhibitor, which is associated with an impaired transcription in active T cells and thus blocks interleukin 2 (IL-2) activation, greatly improved transplantation graft survival [8]. In 1972, Ballinger and Lacy [9] demonstrated that chemical diabetes could be "cured" in mice with islet transplantation.

Many groups had been working in rodent and canine models to perfect islet isolation and maintain demonstrable β-cell insulin secretion [10,11]. Experiments in rats and mice showed that success in islet transplantations could be obtained in rodents [8] and the transplanted islets had a biphasic insulin secretory response to glucose [12]. Islet allotransplantation in animals was also successfully performed [13,14] and it has been possible to isolate human islets that would function in diabetic nude mice [15,16].

The results of these studies highlighted the need for purified islets and the importance of the site of transplantation. Multicellular islet tissue comprises 1.5% by weight of the whole pancreas [17]. Careful digestion is necessary to achieve a purified preparation, and collagenase became the mainstay in the digestion [10]. Success with isolation from human pancreases was more problematic than in animal studies [18,19]. Methods used for the dissociation of islets from the exocrine tissue required a combination of mechanical and enzymatic techniques.

The site of transplantation was also an issue. The pancreatic bed, because of the risk of inducing acute pancreatitis, was not a preferred option. Liver, spleen, renal subcapsule and an omental pouch have been the primary locations tried [14,20–22]. Work with immune privileged sites, such as the testes, has been ongoing but has not been routinely adapted [23]. A site with portal drainage has the natural advantage of mimicking the normal route of insulin delivery and is most commonly used for human islet transplantation [24]. The splenic site was associated with infarction [25] and so the liver, renal and omental pouch have been the preferred areas for islet transplantation. Compared to the native islet bed, all sites have the disadvantages that oxygenation is lower [26] and the intra-islet blood pressure is elevated [27]. Vascularization of the liver is advantageous and may help in the angiogenesis of the islets while an advantage of the omental pouch or renal site is the ability to remove the islets for histologic examination. When the liver is used, an unresolved question is whether the venous drainage of the islets appear in the portal sinusoids or the systemic circulation. A possible drawback of the portal site is that it allows more exposure to high levels of immunosuppressive drugs and their potential toxicity as they are absorbed [28].

Early human studies

The first human islet transplants were performed in the 1970s [29], with insulin independence rarely reported [30]. Up to 1998, approximately 260 patients with T1DM had received an islet transplant, with only 12% remaining insulin independent for more than 1 week [24]. The first two patients, transplanted as part of an early cohort transplanted in Edmonton in 1989 [31], received approximately 260 000 islets at the same time as a renal transplantation. Exogenous insulin was used intravenously for 14 days with intensive glucose monitoring in order to maintain euglycemia, which may help preserve β-cell function [32]. The immunosuppression therapy included corticosteroids, azathioprine, CsA, and Minnesota antilymphocyte globulin. Both patients demonstrated positive C-peptide status post-transplant, but both developed cytomegalovirus infection and lost islet mass, never achieving insulin independence. The subsequent five

patients had transplants using both fresh and cryopreserved islets so that the total islet mass given exceeded 10 000 islet equivalents per kilogram. One patient obtained insulin independence for 2 years [33]. One patient who was transplanted at this time had a liver transplant with partially purified islets infused. Complete portal vein thrombosis ensued necessitating an urgent repeat liver transplant [34]. Another patient attained insulin independence for a period of time but eventually all patients required insulin again. On long-term follow-up, two of these patients have continued C-peptide production, the longest being more than 9 years since her transplant; however, subsequent technical problems arose in Edmonton, particularly with the collagenase, and so purified islets could not be obtained and thus islet transplantation lapsed as an avenue for treating diabetes. Other major centers, particularly Miami/Pittsburg, St. Louis and Milan, also reported early success [35–38] and demonstrated that the transplanted cells may survive for a prolonged time [39,40].

Pancreas transplantation

With improved immunosuppression the possibility of performing whole pancreas transplants, particularly at the time of renal transplantation for end-stage diabetic nephropathy, became a possibility [41]. Initial efforts were associated with peritonitis from exocrine drainage of the pancreatic duct. These problems were surmounted by using bladder drainage [42] such that success rates reached over 80% for 1-year graft survival accompanied by low mortality rates [43–46]. With newer techniques of anastomosis connecting the transplanted duodenum to an enteric drainage site [47], fewer problems were encountered (especially with the acidosis secondary to bicarbonate loss in the urine associated with the bladder anastomosis [48]). More than 25 000 whole pancreas transplants have been carried out worldwide. The 1-year graft survival rate has improved as a result of reduction in technical and immunologic failure rates [49]; however, the overall 10-year graft survival rate for deceased donor pancreas transplants has not substantially improved over time and was 48% for transplants between 1995 and 1999 [50]. Graft survival is better for simultaneous pancreas–kidney transplantation than either pancreas transplant alone or pancreas transplantation after kidney transplantation. The side effects are diminishing but it remains a technically challenging surgical procedure [51–53] with some morbidity and mortality. The excellent glycemic control [54] can lead to reversal of diabetic renal lesions [55], stabilization or improvement in neuropathy [56,57], vascular status [58,59] and stabilization but not necessarily improvement of retinopathy [60,61].

Islet transplantation in the new millennium

The unimpressive results of islet transplantation in the late 1990s, as illustrated by low rates of insulin independence [39,62,63], were related to the islet preparation (purity of preparations and adequate islet numbers) [64] and immunosuppression (potency and toxicity especially in terms of glucose tolerance), as has been reviewed by Hering and Ricordi [65]. The field of islet transplantation was rejuvenated with our report of seven consecutive cases, achieving insulin independence with islet transplantation using a steroid-free immunosuppression regimen (Edmonton protocol) [66]. Some of the factors associated with this success are discussed below.

Islet isolation

Islet mass availability remained a central issue for success. An adequate islet mass contributed to the success of the Edmonton protocol, with >11 000 islet equivalents per kilogram recipient body weight being transplanted.

The normal pancreas has 1.0–1.7 million islets [67,68], yet early studies showed only 250 000 islets being recovered for allotransplantation. Minimizing warm and cold ischemia time and other donor issues are important [69,70]. Using the University of Wisconsin perfusate solution at the time of organ retrieval enhanced the yield [71]. In addition, the intraductal delivery of collagenase [18,19,72], particularly with improved collagenase preparations [73,74], further enhanced the number of islets obtained. Great strides were made in characterizing the collagenase necessary for purified islet isolation, resulting in much better preparations. The enzyme preparation Liberase was widely used for islet isolation and is a blend of primarily collagenase type 1 and 2, but also has collagenase 3 and 4, clostripain, thermolysin and proteases (trypsin, chymotrypsin and elastase), and is associated with a low endotoxin load [74].

Another major advance was the development of a metal chamber by Ricordi *et al.* [75] allowing disassociation of islets with mechanical digestion and the continuous removal and harvesting of the liberated islets [76]. Purification of islets using a refrigerated COBE centrifuge and Ficoll gradient is also important. While it leads to some loss of cells, it reduces the otherwise substantial risk of intraportal hypertension associated with the infusion of unpurified preparations [34,77]. The increased purification may also have a negative effect because ductal elements, which may be important as a source for islet neogenesis, are removed [78].

Short-term culture of the islets may lead to enhanced purity without excessive loss of islets. In addition, the newer methods of islet isolation have removed all xenoproteins from the process, and this could further reduce the risk of rejection.

Immunosuppression

The importance of the appropriate immunosuppression regimen is demonstrated by the fact that with autotransplantation once 2500 islet equivalents per kilogram are provided then insulin independence can usually be achieved; however, three or four times this number of cells are required for insulin independence in the allotransplant environment [79]. In the Edmonton protocol, daclizumab was used for induction, followed by maintenance

immunosuppression therapy with sirolimus (target trough levels of 12–15 μg/L for 3 months, then 10–12 μg/L) and low-dose tacrolimus (target trough levels of 3–6 μg/L) [66]. Such a combination allowed the omission of steroids from the regimen, which was a major advantage in the setting of borderline islet mass by reducing β-cell toxicity. Effective blockade of IL-2 with sirolimus, which inhibits T-cell expression and activation, and daclizumab, an antibody to IL-2 receptors, allows inhibition of T-cell activation and provides potent immunosuppression. Sirolimus can result in lipid abnormalities but was not known to affect glucose tolerance [80,81]. Tacrolimus, a more potent calcineurin inhibitor than CsA, is associated with some diabetogenicity [9,82–84] by inhibiting insulin release [85] in a dose-dependent manner [86], a problem shared by its predecessor CsA [87,88]. Hence, part of the rationale for using sirolimus as the mainstay of immunosuppression with low dose tacrolimus was to reduce diabetogenicity of the maintenance immunosuppression regimen.

Islet transplantation today

Following this initial success, more than 650 islet transplants have been performed worldwide using the Edmonton protocol or variants of it, and incorporating newer advances. Indeed, islet transplantation today is quite different from 10 years ago.

Islet preparation

Pancreas preservation

The University of Wisconsin solution (UW) was effective in pancreas preservation, but prolonged storage before islet isolation led to reduced recovery of viable islets [89]. The two-layer system using perflurodecalin (PFC) and UW for whole pancreas preservation was thus advocated for rescuing ischemically damaged pancreases [90]. This was because of the ability to ensure adequate oxygenation to the pancreas during preservation, and the reduction of cold ischemic injury by promoting adenosine triphosphate production [90]. It was also found that preservation in PFC resulted in the upregulation of anti-apoptotic genes and the downregulation of pro-apoptotic genes [91].

Indeed, islet recovery from pancreases preserved with the two-layer system was double that of UW alone [90,92]. Furthermore, PFC preservation resulted in an islet yield from pancreases procured from marginal donors that was sufficient for clinical transplantation in more cases than with UW alone [92]; however, more recently it was shown that there was no significant difference in islet yield or transplantation outcome regardless of whether the two-layer method or UW alone was used [93]. The authors currently use histidine-tryptophan-ketoglutarate solution alone for pancreas preservation, which appears to be equally effective as UW.

Islet culture

Previously, islets were infused into recipients within 2 hours of isolation to reduce the risk of ischemic injury to the islets [66]; however, this gave little time for appropriate quality control measures to be completed and potential recipients had to live near the transplant center. The authors' current practice is to culture islets for up to 72 hours prior to transplantation. This has numerous advantages because the additional time when the islets are in culture allows for the administration of conditioning or other immunosuppressive therapies, can result in improved safety because transplants can be carried out when the entire transplant team is present and allows for better islet characterization before transplant [94]. In addition, the decrease in total tissue volume with culture may reduce the risk of portal vein thrombosis. Furthermore, the use of regional islet processing centers has been advocated as a means of standardizing the islet product, and hence improving transplant outcomes. Islet culture can result in better islet recovery after shipment [95], and islet culture has now become standard and routine practice at most islet transplant centers worldwide.

Enzyme preparation and digestion protocols

In 2007 it became apparent that the crude collagenase extract in Liberase®, a secretion product of *Clostridium histolyticum* bacteria, could have been contaminated with bovine brain infusion extract, as this extract contains high levels of lipid, carbon and nitrogen which apparently facilitates the proliferation and secretory capacity of the bacteria. The specific risk to an islet patient is the possibility of prion transmission from the cow brain extract through the enzyme and into the pancreas organ during digestion of the gland. The estimated risk is currently unknown, but a working number is currently less than one in ten million – in other words exceedingly remote. Since then, most islet isolation centers have switched to an alternative enzyme manufactured by Serva. This is a GMP (good manufacturing practice) grade enzyme where the potential risk of prion transmission should be dramatically lower as no bovine brain extracts are used in the manufacture process. Using high-pressure liquid chromatography and collagenase activity assay, we found that the Serva collagenase is less pure and less potent than Liberase. With modification of our digestion protocols, we have now managed to achieve a high rate of islet isolation success (defined as >300 000 IEQs, >70% viability and <5 mL packed tissue volume) that is superior to historical outcomes from the Liberase era (unpublished data). Specifically, we use different digestion protocols for younger (≤35 years) versus older donors. For younger donors, we use collagenase and neutral protease simultaneously, while for older donors, a higher amount of collagenase initially, followed by sequential digestion with a lower amount of neutral protease was found to be optimal. Others have found that islet isolation outcomes and islet function are similar with the two enzyme blends [96].

Transplant procedure

Once adequate pure islets are prepared the patient is brought to radiology and percutaneous access is established under midazolam and fentanyl sedation. After infiltration of local anesthetic a 22-gauge Cheba needle is advanced under fluoroscopic guid-

ance into the portal vein. Others have used computer tomography (CT) guidance or it is possible to gain access by the transjugular route or with laparoscopy [97]. A guidewire is then inserted into the main portal vein and a catheter is positioned with confirmation by portal venogram. Purified islets are then infused with frequent monitoring of portal pressure. If the portal pressure doubles or rises above 22 mmHg infusion is halted until it resolves, and if it does not resolve the infusion is discontinued. Initially, we used a 60-mL syringe but quickly adopted the use of an intravenous bag which is prepared in the laboratory [98]. This aids aseptic technique and may also pose less shear pressure on the islets and further provides some constant monitoring of portal pressure during islet infusion.

This percutaneous approach can result in the risk of bleeding from the liver, which was seen in the first report. Subsequently, Gelfoam® pledgets and coils were used to seal the catheter tract, and there was no more bleeding seen in the next 28 cases [99]; however, in 2003, there was a spate of post-procedural bleeding (defined as an acute fall in hemoglobin of 20%, associated with free fluid on ultrasound, the need for blood transfusion or surgical intervention for control of bleeding) [99,100]. Since then, the portal tributary cannulation site was plugged with coils, and the tract ablated using tissue glue (Tisseel®) with no further recurrence of bleeds in the next 35 procedures [100]. More recently, Avitene® paste dissolved in radiologic contrast and saline has been used instead to seal the catheter tract. When adequately deployed, this has eliminated bleeding risks and has the practical advantage of being clearly visible during deployment on fluoroscopy.

Before transplant, intravenous insulin and dextrose infusions are started to maintain euglycemia during transplant. Initially, insulin was discontinued after transplantation and was avoided unless hyperglycemia (serum glucose ≥11.1 mmol/L) occurred [66]. Subsequently, this threshold was lowered and insulin was given if pre-meal glucose was >6.0 mmol/L or 2-hour post-meal glucose was >8.0 mmol/L [99].

Recently, it was shown that maintaining euglycemia in the immediate post-transplant period could contribute to better graft survival [101]. Since mid 2005, it has been our policy to maintain euglycemia (serum glucose 4.0–7.0 mmol/L) following transplant by using intravenous insulin (minimum of 1 unit/hour) with dextrose infusions for the first 48 hours, and subcutaneous insulin thereafter.

Intravenous heparin is also infused to keep the pro-thrombin time 70–90 seconds for 48 hours after transplantation to promote engraftment by reducing the immediate blood mediated inflammatory reaction (IBMIR). Heparin is withheld if there is inadequate tract plugging with Avitene (<3 cm in length) until imaging confirms the absence of bleeding.

After the heparin infusion is discontinued, subcutaneous low molecular weight heparin (30 mg enoxaparin twice daily) is administered for 7 days and 81 mg/day aspirin for 14 days.

In addition, patients receive 400 mg sulfamethozazole–80 mg trimethoprim one tablet daily for 6 months, *Pneumocystis* pneumonia prophylaxis and 900 mg/day valganciclovir for 14 weeks when there is cytomegalovirus status mismatch between donor and recipient, and in patients who have received lymphocyte-depleting induction agents.

Immunosuppression

The immunosuppressive regimen of the original Edmonton protocol is still being used today, but with some modifications. Daclizumab was initially given at a dosage of 1 mg/kg every 2 weeks five times. After 2003, this was changed to 2 mg/kg at transplant and at 5 days post-transplant [99]. This was because the latter regimen was found to be efficacious and was more convenient for patients.

Recently, induction with antithymocyte globulin (6 mg/kg) and etanercept, with maintenance immunosuppression with tacrolimus (target trough level 8–10 μg/L) and mycophenolate mofetil (1 g twice daily), has been used [100]. Also, a lymphocyte depletion protocol consisting of alemtuzumab (Campath-1H), tacrolimus and mycophenolate mofetil is being evaluated. Preliminary data suggest that the use of these potent induction agents have improved short to medium-term graft outcomes [101–103].

Many of the initial patients who were on sirolimus and tacrolimus for maintenance immunosuppression have had intolerable side effects which were attributed to sirolimus, necessitating a switch of immunosuppression to tacrolimus and mycophenolate mofetil. This latter combination appears to be as efficacious and better tolerated [104]. Furthermore, sirolimus impairs β-cell regeneration, and could contribute to the observed gradual loss of graft function seen following islet transplantation [105]. Thus, this combination is increasingly the first-line maintenance immunosuppression choice for islet transplantation.

Islet transplantation outcomes

Glycemic control

Insulin independence was achieved in 11 out of the first 12 patients, after a minimum of 9000 IE/kg were transplanted [66]. HbA_{1c} levels improved in all patients, and this was achieved without hypoglycemia and was accompanied by an improved stability of glucose control [66]. Unfortunately, insulin independence was not sustainable in the long term for the majority of patients. From survival analysis, only approximately 10% of patients remained off insulin at 5 years, although most patients (approximately 80%) still had C-peptide present (Figure 61.6) [99].

In terms of overall blood glucose control, patients who remained off insulin did the best (median HbA_{1c} of 6.2%, 44 mmol/mol), similar to the patients who were back on insulin but still had C peptide (median HbA_{1c} of 6.7%, 50 mmol/mol). Patients who had lost all graft function had relatively poor glucose control (median HbA_{1c} of 9.0%, 75 mmol/mol) and required more insulin than before the transplant (Figure 61.7) [99].

The HYPO score and lability index (LI) were developed to measure the severity of hypoglycemia and glycemic lability. The HYPO score is generated using a combination of 4 weeks of self glucose monitoring results and the patients' self-reported hypoglycemic episodes over the previous year. For every episode of hypoglycemia <3 mmol/L during the 4 weeks, patients were instructed to record all symptoms felt and whether assistance was required for recognition or treatment of the hypoglycemia. Higher scores were given for more severe hypoglycemic episodes (i.e. lower glucose values, absence of symptoms or neuroglycopenic symptoms, needing outside help for recognition/treatment), while from the self-reported episodes during the past year higher points were awarded if an ambulance was called or glucagon given. The LI was also calculated from the 4 weeks of glucose records, using a formula that takes the number of glucose readings, the glucose values and the time interval between testing into account [106]. These scores are now routinely used in the assessment of suitability of a candidate for transplant, as well as for follow-up of patients after transplant. In addition, the Clarke score [107] is also frequently used, with a score of four or more indicating hypoglycemia unawareness.

Both the HYPO score and LI show marked improvement post-transplant. With resumption of insulin use, there was more lability and some episodes of hypoglycemia, but both scores were still better than pre-transplant [99].

A key indication for islet transplantation is hypoglycemia unawareness. Patients who have received a pancreas transplant have restoration of their counter-regulatory response to hypoglycemia [108,109]. The autonomic response and hence hypoglycemia awareness is also improved post-pancreas transplant [110]; however, the same restoration in counter-regulatory responses and symptom recognition was not seen following successful islet transplantation [111]. Conversely, others have found that counter-regulatory hormonal and symptom responses to hypoglycemia do improve after islet transplantation [112–114]. The reasons for these differences are unclear; however, there may be a subset of patients who have return of hypoglycemia awareness, and/or the timing of testing could be critical. Indeed, we have noted that 85% of our cohort of islet transplant recipients reported return of symptoms of hypoglycemia, although 62% of them subsequently lost hypoglycemia awareness.

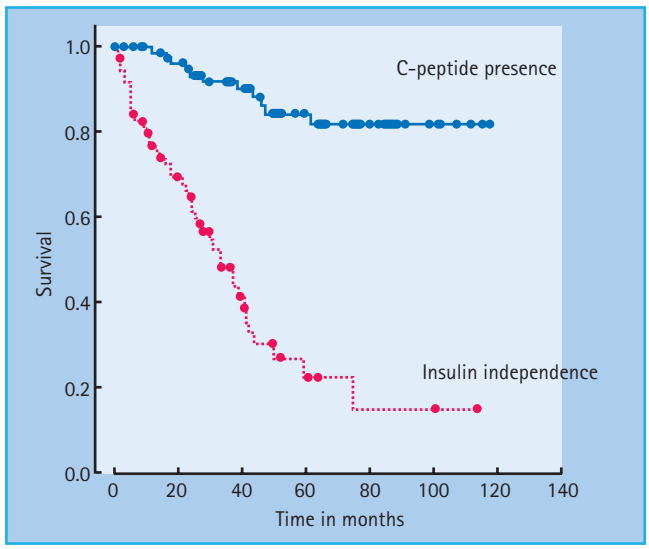

Figure 61.6 Current survival analysis for insulin independence and graft function as indicated by presence of C-peptide.

Diabetes complications

Retinopathy

Retinopathy has been reported to remain stable following islet transplantation [115,116]. The majority of our patients (approxi-

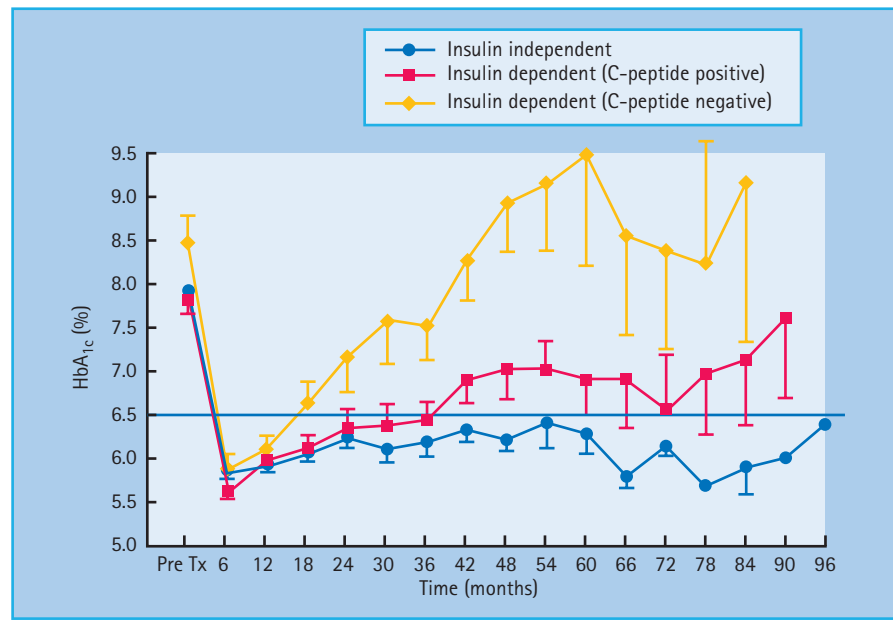

Figure 61.7 Glycemic control is related to islet graft function. DCCT result (%) = (0.0915 × New IFCC result in mmol/mol) + 2.15.

mately 80%) showed either no change or an improvement in retinopathy grade compared with baseline 3 years following islet transplantation; however, in our cohort, 18 of 98 (18.4%) subjects had either vitreous hemorrhage or need for laser photocoagulation after islet transplantation, suggesting that close ophthalmologic follow-up remains necessary.

Nephropathy

We have previously reported that the estimated glomerular filtration rate (eGFR) (by MDRD study equation) declined with time. The median rate of eGFR decline was −0.39 mL/min/1.73 m^2/month, with wide inter-patient variability [117]. This decline in GFR is comparable to that seen in optimally treated albuminuric patients with T1DM [118]; however, for one-third of the patients, the decline in eGFR exceeded that in untreated diabetic nephropathy [119]. In addition, on follow-up, progression in albuminuria was seen in 24% of the patients, with regression only in 2.4% [117].

When tacrolimus and mycophenolate mofetil were used for maintenance immunosuppression, no difference in the rate of GFR decline was seen compared with either medically treated controls or the general population, nor was there any progression in albuminuria [120].

It is not clear at present whether the decline in renal function in islet alone transplants is brought about by progression of diabetic nephropathy or the effects of immunosuppression, in particular the combination of sirolimus and tacrolimus. Nevertheless, care has to be taken during the patient selection process with regard to the assessment of renal function.

Neuropathy

There was no change in neuropathy status as assessed by vibration perception threshold or neuropathy disability score in our cohort [121], and others have shown stabilization of neuropathy [115].

Cardiovascular disease

A substantial proportion (30%) of islet transplant recipients at our center have pre-existing coronary artery disease (CAD) prior to transplantation. In terms of CAD risk factors, triglyceride levels increased (0.82 ± 0.04 vs 1.09 ± 0.06 mmol/L; $P < 0.001$), while there was a reduction in low density lipoprotein (LDL) cholesterol levels (2.53 ± 0.06 vs 2.14 ± 0.06 mmol/L; $P < 0.001$), likely from an increase in statin use following transplantation. Blood pressure remained unchanged. Following islet transplantation, the rate of incident (new or worse) CAD was similar to the general population with T1DM at 8.9 events/1000 patient years [122].

Procedure-related complications

Complete portal vein thrombosis has not occurred with the use of purified islet allograft preparations, although partial thrombosis (of right or left branch, or peripheral segmental vein) was seen in about 5% of patients, all of whom were treated with anticoagulation without any long-term clinical sequelae [66,99]. The risk of portal vein thrombosis has been minimized by limiting the islet packed cell volume, careful monitoring of portal pressures during islet infusion and intraportal dosing with heparin followed by systemic anticoagulation. Other rare complications include gallbladder puncture and arteriovenous fistulae formation.

Liver enzymes (aspartate transaminase, alanine aminotransferase and alkaline phosphatase) were elevated in more than 50% of transplants and are generally subclinical. These enzymes peak at around 1 week and resolve spontaneously within a month [123].

Changes consistent with fatty liver have also been observed on imaging post-transplantation [124]. These steatotic changes were confirmed with biopsy in some cases, and may be related to the high insulin levels the hepatocytes are exposed to following intrahepatic transplant [124]. It is currently unclear whether these changes may result in any long-term sequelae.

Side effects of immunosuppression

Most patients may have some side effects from immunosuppresion, but it is highly variable among patients.

Mouth ulcers occur in >90% of the patients and is associated with sirolimus. These are usually small and self-limiting, but on occasion may be numerous and/or severe enough to require inpatient care. Most patients respond to topical therapy, dose reduction and switching to the tablet formulation of sirolimus [99].

Gastrointestinal disturbances, either constipation or diarrhea, are also common, occurring in 60% of the patients, while acne was noted in 52%. Peripheral edema was reported by 43% of the patients [100].

Ovarian cysts following transplants have been found to be common [99]. Another study found that among women, ovarian cysts occurred in 62% of the subjects, and menstrual irregularity developed in all six subjects who had regular menstrual cycles before transplant [125]. At our center, new ovarian cysts were found in 33 of 57 (57.9%) women after islet transplantation. Most cysts were asymptomatic but 14 women reported pelvic pain. Sirolimus withdrawal was associated with a reduction in cyst size and resolution of cysts in 80% of the subjects. Also, the use of combined oral contraception appeared to be protective against ovarian cyst development [126].

Other complications related to immunosuppressive therapy include anemia, leukopenia, hypertension, dyslipidemia, weight loss and fatigue [100].

Tacrolimus was associated in a dose-dependent manner with tremor and nephrotoxicity. The combination of sirolimus and tacrolimus could also worsen renal function. Indeed, two patients in whom tacrolimus was switched to mycophenolate mofetil had stabilization of renal impairment [127]. Also, three patients had resolution of proteinuria after sirolimus was withdrawn and replaced by a combination of mycophenolate mofetil and higher dose tacrolimus [128].

Mycophenolate mofetil is generally well tolerated, with the most commonly reported side effect being gastrointestinal in

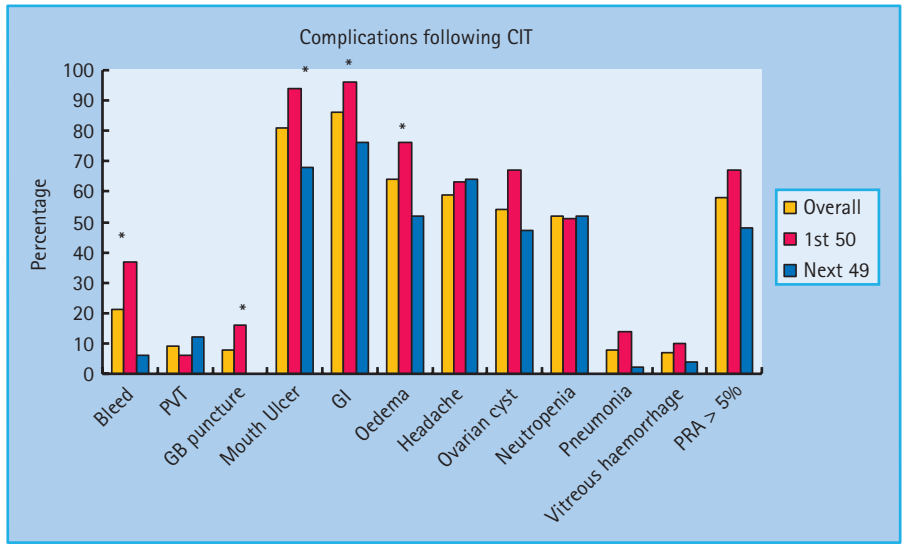

Figure 61.8 Complications following clinical islet transplantation – first 50 vs next 49 patients in Edmonton. * Indicates significant difference ($P < 0.05$) between first 50 and next 49 subjects. GB, gall bladder; GI, gastrointestinal; PRA, panel reactive antibody; PVT, portal vein thrombosis.

nature (bloating, diarrhea, abdominal cramps), which usually subside with dose reduction [129].

Pneumonia occurred in three patients, of whom one was considered fungal in etiology [98].

Cytomegaloviral disease has not occurred, although seroconversion from negative to positive has occurred in 4 of 67 (6%) patients. To date, no lymphoproliferative disease has been observed.

With experience, we have learned to tailor the immunosuppression regimen and target drug levels to minimize side effects without compromising graft function, such that fewer complications are seen in patients transplanted more recently than in earlier patients (Figure 61.8).

A total of 34 patients had undergone immunosuppression change from sirolimus + tacrolimus to tacrolimus + mycophenolate mofetil following islet transplantation. The three most frequent reasons for immunosuppression change were peripheral edema (18/34, 53%), gastrointestinal symptoms (11/34, 32%) and ovarian cysts in women (9/26, 35%). These all improved after the immunosuppression change. Also noteworthy, there were no changes in graft function or immune status following the change (Table 61.1) [104].

Table 61.1 Reasons for and outcomes after immunosuppression (IS) change.

	No. of patients with event before IS change (n = 34)	% with improvement / no change / worsening after IS change	*P* value
Peripheral edema	18	83 / 17 / 0	<0.001
Gastrointestinal symptoms	11	75 / 8 / 17	0.01
Fatigue	9	44 / 55 / 0	0.05
Proteinuria/renal function decline	6	100 / 0 / 0	0.01
Ulcers	4	100 / 0 / 0	0.05
Ovarian cysts/menstrual abnormalities in female patients (n = 26)	9	78 / 22 / 0	0.01

Immune sensitization

The percentage of panel reactive antibodies (PRA) increased in 13% of the patients from levels <15% to ≥15% after transplant [99]. Recently, it has become clear to us that positive PRA does impact islet transplant outcome negatively. Indeed, pre-transplant PRA of >15% in either class I or II is an independent predictor of poor graft survival (in terms of C-peptide) after transplant [130].

For individuals with high PRA, it will be important to determine the specific antibodies causing the positive PRA and a flow cytometry-based cross-match performed against potential donors before transplant. Our current policy is to perform prospective cross-matches for all recipients with PRA above 5% and for those who have received previous transplants.

It has become apparent that high rates of broad PRA sensitization were observed in patients when immunosuppression was slowly and completely withdrawn following complete graft loss [131]. Our current approach is therefore not to withdraw all immunosuppression when a patient loses all islet function, but rather to wean down to single agent therapy with mycophenolate (Myfortic® therapy). We currently plan to continue this for at least 2 years after function is lost in order to reduce the risk of subsequent sensitization. The effectiveness of this strategy is still to be evaluated.

Indications and contraindications for islet transplantation

At our center, islet transplantation is offered to patients with T1DM who have severe hypoglycemia and/or hypoglycemia unawareness, or glycemic lability, in spite of optimal medical therapy with frequent blood glucose monitoring and the use of multiple daily insulin injections or continuous subcutaneous insulin infusion. This is, by and large, still based on clinical judgment, although the use of the HYPO score and LI adds an objective component to the decision. Some patients have progressive complications despite optimization of medical therapy. Although we originally considered this group, they are now the exception, as we are concerned about the potential for immunosuppression to exacerbate renal impairment.

Diabetes-related complications are not absolute contraindications for islet transplantation. For patients with unstable retinopathy, we recommend waiting for 6 months from the last treatment for the disease to stabilize before islet transplantation because there is a risk of worsening following transplantation. The sirolimus and tacrolimus combination is avoided in patients with macroalbuminuria (>300 mg/day). We prefer not to transplant patients with mild to moderate reduction in GFR (<60 mL/min/1.73 m^2). We also recommend waiting for at least 6 months following myocardial infarction, revascularization procedure or evidence of ischemia on functional cardiac testing. A severely reduced left ventricular ejection fraction <30% is a contraindication for islet transplantation.

The current accepted indications and contraindications for islet transplantation in most islet transplantation centers are listed in Table 61.2.

Table 61.2 Indications and contraindications for islet transplantation.

Indications for islet transplantation

Clinical history compatible with type 1 diabetes, with stimulated C-peptide <0.3 μg/L on mixed meal tolerance test

Intensive diabetes management:

- Glucose testing ≥3 times/day
- ≥3 insulin injections/day or insulin pump as directed by endocrinologist, diabetologist or diabetes specialist with ≥3 clinical evaluations during the past year

≥1 severe hypoglycemic event, defined as an event with one of the following symptoms: memory loss; confusion; uncontrollable behavior; irrational behavior; unusual difficulty in awakening; suspected seizure; seizure; loss of consciousness; or visual symptoms, in which the subject was unable to treat him/herself and with blood glucose <54 mg/dL (3 mmol/L) or prompt recovery after oral carbohydrate, intravenous glucose or glucagon in the past 1 year

One of the following:

- Reduced hypoglycemia awareness (Clarke score ≥4 or HYPO score ≥90th percentile or ≥1047) within last 6 months
- Marked glycemic lability with wide swings in glucose levels despite optimal therapy, with glycemic lability index ≥90th percentile or 433 within last 6 months
- Composite Clarke score ≥4 + HYPO score ≥75th percentile (≥423) + lability index ≥75th percentile (≥329)

Contraindications for islet transplantation

Glycated hemoglobin ≥10% (86 mmol/mol)

Untreated proliferative retinopathy

Blood pressure >160/100 mmHg

Glomerular filtration rate <80 mL/min/1.73 m^2

Presence or history of macroalbuminuria >300 mg/day

Presence or history of panel reactive anti-HLA antibodies (by flow cytometry)

Active infections, including:

- Hepatitis B, hepatitis C or HIV
- Tuberculosis requiring treatment within the previous 3 years
- Invasive aspergillus, histoplasmosis or coccidiomycosis within 1 year

Severe cardiac disease:

- Myocardial infarction within 6 months
- Evidence of ischemia on functional cardiac testing within 1 year
- Left ventricular ejection fraction <30%

Any history of malignancy except for completely resected squamous or basal cell carcinoma of the skin

A history of factor V deficiency

Any coagulopathy or medical condition requiring long-term anticoagulant therapy after transplantation

Receiving treatment for a medical condition requiring chronic use of systemic steroids except for the use of ≤5 mg/day prednisolone or equivalent

Any medical condition that could interfere with safe participation in islet transplantation

Desired pregnancy (female recipient)

Patient evaluation

At the assessment visit for islet transplantation the patient should have a realistic expectation of the outcome. If the patient has frequent hypoglycemia and glycemic lability problems, then both of these are readily correctable by islet transplantation. The ability to render the patient free of the progression risk of diabetes complications is unproven at this time, although given good glycemic control this may be expected in the longer term. Patients who have active infection, a history of cancer, severe vascular disease or active foot ulceration, who abuse alcohol or drugs, or who are younger than 18 or older than 65 years are not usually considered. Once the patient meets the entry criteria and is willing to accept the risks of the procedure and immunosuppression, a full evaluation is required. This includes a thorough history and physical examination, the latter concentrating on diabetes complications – retinopathy, neuropathy (autonomic and peripheral) and vascular disease. Our laboratory evaluation includes details of these complications with an ophthalmology report required, 24-hour urine for albuminuria, protein and creatinine clearance; together with a serum creatinine, ECG, stress methoxyisobutylisonitrile (MIBI) scan and a lipid panel. If there is any suggestion of an abnormality either clinically or on vascular testing, coronary angiogram is performed. In addition, the basic transplant screens are required, including blood group, complete blood count, coag-

ulation screen, liver function tests, electrolytes, calcium, magnesium, phosphorus, checks for HIV, hepatitis, Epstein–Barr virus, syphilis, cytomegalovirus and urine culture. An ultrasound of the abdomen and liver is required to ensure that no lesions are present in the liver. A hemangioma on the right side of the liver would place a patient at increased risk of bleeding during the procedure if a percutaneous approach was used. In subjects older than 40 years, mammograms are performed in women and prostate-specific antigen determinations in men. Considerable time is spent reviewing the potential complications with the patients so that each individual can make a personal assessment of the risk:benefit ratio for themselves and decide if they wish to proceed. In most other transplant settings (heart and liver), the issues are life and death but in islet transplantation this is not the case, as continuing to work with other insulin regimens is possible and thus risk:benefit issues are different.

Challenges and future directions

Islet shortage

The shortage of donor pancreases for islet transplantation remains a challenge. Most patients require more than one islet infusion to become insulin independent. Given the limited supply of organs, the ability to achieve insulin independence after infusion of islets from a single donor is an important goal which has been achieved by Hering *et al.* [101] in carefully selected recipients given excellent islet preparations, intensive peri-transplant management and alternative induction immunosuppression. Other alternative sources of islets include xenografts (e.g. porcine islets) or stem cells, but remain in the preclinical experimental phase at present [132–134].

Islet engraftment

Defects in insulin secretion soon after transplantation of what should be an adequate islet mass indicates that many islets were lost at the time of engraftment. This could be because of hypoxia early post-transplant [135], the toxic effects of the immunosuppressive drugs [136] and the IBMIR, which results in immune destruction of islets [137]. It has been estimated that only one-third of islets engraft successfully [138].

Various strategies are being tested in attempts to improve engraftment, including the use of vascular growth factors to promote revascularization [139], inhibitors of IBMIR [140] and anti-apoptotic peptides (e.g. caspase inhibitors) [141]. The use of low molecular weight dextran sulfate was found to block IBMIR to a greater extent than heparin, possibly by its more potent inhibition of the complement system [142]. The use of islet surface heparinization was also shown to attenuate IBMIR significantly *in vitro* and *in vivo*, without systemic side effects [143].

Monitoring the islet graft

A key barrier to understanding what happens to the islet graft after transplantation is the lack of access to the graft. Current methods of monitoring graft function are based on the measurement of markers of glucose homeostasis, which may not be disrupted in early stages of rejection [144]. Immunologic monitoring is limited by the lack of standardized markers for autoimmunity and rejection [145]. Unlike other solid organ transplants, serial protocol liver biopsies may not yield sufficient islet tissue for examination. Several approaches for β-cell imaging have been proposed, but are still in various stages of development [146]. Clearly, it is of vital importance to develop one or more methods to detect graft dysfunction in its early stages or even before it happens, so as to allow intervention in an attempt to rescue the graft.

Promoting graft survival

Islet graft function appears to decline over time, with most patients returning to insulin use. The glucagon-like peptide 1 (GLP-1) agonist, exenatide, when used in islet transplant recipients with failing islet graft function, resulted in reduction in insulin requirements, but this could not be sustained when the drug was discontinued, suggesting that there was no trophic effect on β-cell mass [147]. The lack of detectable changes in markers of allo-immunity or auto-immunity in the majority of islet transplant recipients, together with the absence of significant inflammatory infiltrate in histologic specimens of islet transplants, suggest that non-immunologic mechanisms have an important role in the gradual graft loss [99,148]. Possible culprits include increased metabolic demand and toxicity of immunosuppressants, and certainly warrants further investigation.

Immunosuppression toxicity

While newer immunosuppressant drug combinations are associated with less toxicity, the ideal situation would be the ability to withdraw immunosuppressive drugs after an initial period of use post-transplant. To achieve this, tolerance to the graft must be induced. Co-stimulation blockade has shown promise for tolerance induction. There have been encouraging results with belatacept (LEA29Y), a potent new CTLA4-Ig in primate models of islet transplants [149], and clinical trials in human subjects are being undertaken. Indeed, the use of belatacept for ongoing maintenance immunosuppression, thus allowing the avoidance of calcineurin inhibitors, has been used successfully in the renal transplantation setting and may be the basis for future maintenance therapy [150].

Another strategy would be islet encapsulation, but this has been associated with limited success to date [151].

Conclusions

Islet transplantation can correct problems with glycemic lability and recurrent hypoglycemia. Given its technical ease, it is particularly suitable for those with problems with glycemic control and no other major complications. The more technically difficult whole pancreas transplant provides stable glucose control and is

ideal in those undergoing simultaneous renal transplant. The islet transplant procedure has some risks, both acutely (particularly bleeding, and thrombosis in the portal vein circulation) and in the long-term, the unknown but real risk of sepsis and neoplasms. For some patients with major problems, with diabetes control these risks are acceptable. Whether the good glycemic control attained will prevent complications in the long term will take years to resolve. Using the indication of progressive diabetes complications is less suitable at this time, given the problems encountered. Islet transplantation can free a patient with very difficult diabetes from the risks of frequent hypoglycemia or glycemic lability. The decision whether to proceed can only be made by an informed patient who has to cope with difficult diabetes on a daily basis.

Major steps have been taken in islet transplantation but more needs to be done. Significant changes over the past 10 years have resulted in improved outcomes, but many challenges still remain. Islet transplantation has faced hurdles before and overcome them. These new challenges can be met and solved. To quote Sir Winston Churchill, "Now this is not the end. It is not even the beginning of the end. But it is, perhaps, the end of the beginning."

References

1 Banting FG, Best CH, Collip JB, Campbell WR, Fletcher AA. Pancreatic extracts in the treatment of diabetes mellitus: preliminary report. *CMAJ* 1922; **12**:141–146.

2 Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993; **329**:977–986.

3 UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 1998; **352**:837–853.

4 Reeves ML, Seigler DE, Ryan EA, Skyler JS. Glycemic control in insulin dependent diabetes mellitus: comparison of outpatient intensified conventional therapy with continuous subcutaneous infusion. *Am J Med* 1982; **72**:673–680.

5 Tsui E, Barnie A, Ross S, Parkes R, Zinman B. Intensive insulin therapy with insulin lispro: a randomized trial of continuous subcutaneous insulin infusion versus multiple daily insulin injection. *Diabetes Care* 2001; **24**:1722–1727.

6 Ratzmann KP, Bruns W, Schulz B, Zander E. Use of the artificial B-cell (Biostator) in improving insulin therapy in unstable insulin-dependent diabetes. *Diabetes Care* 1982; **5**:11–17.

7 Williams P. Notes on diabetes treated with extract and by grafts of sheep's pancreas. *Br Med J* 1894; **2**:1303–1304.

8 Pirsch JD, Miller J, Deierhoi MH, Vincenti F, Filo RS. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. *Transplantation* 1997; **7**: 977–983.

9 Ballinger WF, Lacy PE. Transplantation of intact pancreatic islets in rats. *Surgery* 1972; **72**:175–186.

10 Lindall A, Steffes M, Sorensen R. Immunoassayable insulin content of subcellular fractions of rat islets. *Endocrinology* 1969; **85**: 218–223.

11 Lacy PE, Walker MM, Fink CJ. Perifusion of isolated rat islets *in vitro*: participation of the microtubular system in the biphasic release of insulin. *Diabetes* 1972; **21**:987–998.

12 Bromme HJ, Hahn HJ, Blech W. Biphasic release of insulin from islets of Langerhans after their transplantation into the liver of rats. *Horm Metab Res* 1998; **20**:138–140.

13 Bowen KM, Lafferty KJ. Reversal of diabetes by allogenic islet transplantation without immunosuppression. *Aust J Exp Biol Med Sci* 1980; **58**:441–447.

14 Naji A, Silvers WK, Plotkin SA, Dafoe D, Barker CF. Successful islet transplantation in spontaneous diabetes. *Surgery* 1979; **86**:218–226.

15 Ricordi C, Scharp DW, Lacy PE. Reversal of diabetes in nude mice after transplantation of fresh and 7-day culture (24°C) human pancreatic islets. *Transplantation* 1988; **45**:994–996.

16 Gerling IC, Kotb M, Fraga D, Sabek O, Gaber AO. No correlation between *in vitro* and *in vivo* function of human islets. *Transplant Proc* 1998; **30**:587–588.

17 Hellman B. The frequency distribution of the number and volume of the islets of Langerhans in man. *Acta Soc Med Ups* 1959; **64**:432-60.

18 Rajotte RV,Warnock GL, Evans MG, Ellis D, Dawidson I. Isolation of viable islets of Langerhans from collagenase-perfused canine and human pancreata. *Transplant Proc* 1987; **19**:918–922.

19 Gray DWR, McShane P, Grant A, Morris PJ. A method for isolation of islets of Langerhans from the human pancreas. *Diabetes* 1984; **33**:1055–1061.

20 Marchetti P, Scharp DW, Olack BJ, Swanson CJ, Bier D, Cobelli C, *et al.* Glucose metabolism, insulin sensitivity, and glucagon secretion in dogs with intraportal or intrasplenic islet autografts. *Transplant Proc* 1992; **24**:2828–2829.

21 Merani S, Toso C, Emamaullee J, Shapiro AM. Optimal site for pancreatic islet transplantation. *Br J Surg* 2008; **95**:1449–1461.

22 Yasunami Y, Lacy PE, Finek EH. A new site for islet transplantation: a peritoneal–omental pouch. *Transplantation* 1983; **36**:181–182.

23 Selawry HP, Whittington K. Extended allograft survival of islets grafted into intra-abdominally placed testis. *Diabetes* 1984; **33**:405–406.

24 White SA, James RFL, Swift SM, Kimber RM, Nicholson ML. Human islet cell transplantation: future prospects. *Diabet Med* 2001; **18**:78–103.

25 White SA, London NJ, Johnson PR, Davies JE, Pollard C, Contractor HH, *et al.* The risks of total pancreatectomy and splenic islet autotransplantation. *Cell Transplant* 2000; **9**:19–24.

26 Carlsson PO, Palm F, Andersson A, Liss P. Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site. *Diabetes* 2001; **50**:489–495.

27 Carlsson PO, Jansson L, Andersson A, Källskog O. Capillary blood pressure in syngeneic rat islets transplanted under the renal capsule is similar to that of the implantation organ. *Diabetes* 1998; **47**:1586–1593.

28 Shapiro AM, Gallant H, Hao E, Wong J, Rajotte R, Yatscoff R, *et al.* Portal vein immunosuppressant levels and islet graft toxicity. *Transplant Proc* 1998; **39**:641.

29 Najarian JS, Sutherland DER, Matas AJ, Steffes MW, Simmons RL, Goetz FC. Human islet transplantation: a preliminary report. *Transplant Proc* 1977; **9**:233–236.

30 Largiader F, Kolb E, Binswanger U, Illig R. Successful allotransplantation of an island of Langerhans. *Schweiz Med Wochenschr* 1979; **109**:1733–1736.

31 Warnock GL, Kneteman NM, Ryan EA, Evans MG, Seelis RE, Halloran PF, *et al.* Continued function of pancreatic islets after transplantation in type 1 diabetes. *Lancet* 1989; **2**:570–572.

32 Diabetes Control and Complications Trial Research Group. Effect of intensive therapy on residual β-cell function in patients with type 1 diabetes in the Diabetes Control and Complications Trial. *Ann Intern Med* 1998; **128**:517–523.

33 Warnock GL, Kneteman NM, Ryan EA, Rabinovitch A, Rajotte RV. Long-term follow-up after transplantation of insulin-producing pancreatic islets into patients with type1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1992; **35**:89–95.

34 Shapiro AMJ, Lakey JRT, Rajotte RV, Warnock GL, Friedlich MS, Jewell LD, *et al.* Portal vein thrombosis after transplantation of partially purified pancreatic islets in a combined human liver/islet allograft. *Transplantation* 1995; **59**:1060–1063.

35 Ricordi C, Tzakis A, Alejandro R, Zeng YJ, Demetris AJ, Carroll P, *et al.* Detection of pancreatic islet tissue following islet allotransplantation in man. *Transplantation* 1991; **52**:1079–1080.

36 Tzakis AG, Ricordi C, Alejandro R, Zeng Y, Fung JJ, Todo S, *et al.* Pancreatic islet transplantation after upper abdominal exenteration and liver replacement. *Lancet* 1990; **336**:402–405.

37 Scharp DW, Lacy PE, Santiago JV, McCullough CS, Weide LG, Boyle PJ, *et al.* Results of our first nine intraportal islet allografts in type 1, insulin-dependent diabetic patients. *Transplantation* 1991; **51**:76–85.

38 Socci C, Davalli AM, Vignali A, Bertuzzi F, Maffi P, Zammarchi O, *et al*. Evidence of *in vivo* human islet graft function despite a weak response to *in vitro* perfusion. *Transplant Proc* 1992; **24**: 3056–3057.

39 Alejandro R, Lehmann R, Ricordi C, Kenyon NS, Angelico MC, Burke G, *et al*. Long-term function (6 years) of islet allografts in type 1 diabetes. *Diabetes* 1997; **46**:1983–1989.

40 Robertson RP, Lanz KJ, Sutherland DE, Kendall DM. Prevention of diabetes for up to 13 years by autoislet transplantation after pancreatectomy for chronic pancreatitis. *Diabetes* 2001; **50**:47–50.

41 Kelly WD, Lillehei RC, Merkel FK, Idezuki Y, Goetz FC. Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. *Surgery* 1967; **61**:827–837.

42 Cook K, Sollinger HW, Warner T, Kamps D, Belzer FO. Pancreaticocystostomy: an alternative method for exocrine drainage of segmental pancreatic allografts. *Transplantation* 1983; **35**:634–636.

43 Bûsing M, Heimes M, Martin D, Schulz T, Dehof S, Kozuschek W. Simultaneous pancreas-/kidney transplantation: the Bochum experience. *Exp Clin Endocrinol Diabetes* 1997; **105**:92–97.

44 Sutherland DER. Pancreas transplantation as a treatment for diabetes: indications and outcome. In: Bardin CW, ed. *Current Therapy in Endocrinology and Metabolism*, 6th edn. St. Louis: Mosby, 1997: 496–499.

45 Sutherland DE, Gruessner AC, Gruessner RWG. Pancreas transplantation: a review. *Transplant Proc* 1998; **30**:1940–1943.

46 Ryan EA. Pancreas transplants: for whom? *Lancet* 1998; **351**:1072–1073.

47 Kuo PC, Johnson LB, Schweitzer EJ, Bartlett ST. Simultaneous pancreas/kidney transplantation: a comparison of enteric and bladder drainage of exocrine pancreatic secretions. *Transplantation* 1997; **63**:238–243.

48 Nghiem DD, Gonwa TA, Corry RJ. Metabolic effects of urinary diversion of exocrine secretions in pancreatic transplantation. *Transplantation* 1987; **43**:70–73.

49 Gruessner AC, Sutherland DE. Pancreas transplant outcomes for United States (US) and non-US cases as reported to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR) as of June 2004. *Clin Transplant* 2005; **19**:433–455.

50 Waki K, Kadowaki T. An analysis of long-term survival from the OPTN/UNOS Pancreas Transplant Registry. *Clin Transpl* 2007: 9–17.

51 Humar A, Kandaswamy R, Granger D, Gruessner RW, Gruessner AC, Sutherland DER. Decreased surgical risks of pancreas transplantation in the modern era. *Ann Surg* 2000; **231**:269–275.

52 Sutherland DE, Gruessner RW, Gruessner AC. Pancreas transplantation for the treatment of diabetes mellitus. *World J Surg* 2001; **25**:487–496.

53 Sutherland DE, Gruessner RW, Dunn DL, Matas AJ, Humar A, Kandaswamy R, *et al.* Lessons learned from more than 1,000 pancreas transplants at a single institution. *Ann Surg* 2001; **233**:463–501.

54 Robertson RP, Sutherland DE, Lanz KJ. Normoglycaemia and preserved insulin secretory reserve in diabetic patients 10–18 years after pancreas transplantation. *Diabetes* 1999; **48**:1737–1740.

55 Fioretto P, Steffes MW, Sutherland DER, Goetz FC, Mauer M. Reversal of lesions of diabetic nephropathy after pancreas transplantation. *N Engl J Med* 1998; **339**:69–75.

56 Kennedy WR, Navarro X, Goetz FC, Sutherland DER, Najarian JS. Effects of pancreatic transplantation on diabetic neuropathy. *N Engl J Med* 1990; **322**:1031–1037.

57 Martinenghi S, Comi G, Galardi G, Di Carlo V, Pozza G, Secchi A. Amelioration of nerve conduction velocity following simultaneous kidney/pancreas transplantation is due to the glycaemic control provided by the pancreas. *Diabetologia* 1997; **40**:1110–1112.

58 Jukema JW, Smets YFC, van der Pijl JW, Zwinderman AH, Vliegen HW, Ringers J, *et al.* Impact of simultaneous pancreas and kidney transplantation on progression of coronary atherosclerosis in patients with end-stage renal failure due to type 1 diabetes. *Diabetes Care* 2002; **25**:906–911.

59 Fiorina P, La Rocca E, Venturini M, Minicucci F, Fermo I, Paroni R, *et al.* Effects of kidney–pancreas transplantation on atherosclerotic risk factors and endothelial function in patients with uremia and type 1 diabetes. *Diabetes* 2001; **50**:496–501.

60 Wang Q, Klein R, Moss SE, Klein BE, Hoyer C, Burke K, *et al.* The influence of combined kidney–pancreas transplantation on the progression of diabetic retinopathy. *Ophthalmology* 1994; **101**: 1071–1076.

61 Konigsrainer A, Miller K, Steurer W, Kieselbach G, Aichberger C, Ofner D, *et al.* Does pancreas transplantation influence the course of diabetic retinopathy? *Diabetologia* 1991; **34**(Suppl 1):86–88.

62 Brendel MD, Hering BJ, Schulz AO, Bretzel RG. International Islet Transplant Registry Report. *Justus-Liebig-University of Giessen* 1999; 1–20.

63 Benhamou PY, Oberholzer J, Toso C, Kessler L, Penfornis A, Bayle F, *et al.* Human islet transplantation network for the treatment of type 1 diabetes: first data from the Swiss–French GRAGIL consortium (1999–2000). *Diabetologia* 2001; **44**:859–864.

64 Weir GC, Bonner-Weir S, Leahy JL. Islet mass and function in diabetes and transplantation. *Diabetes* 1990; **39**:401–405.

65 Hering B, Ricordi C. Islet transplantation in type 1 diabetes: results, research priorities and reasons for optimism. *Graft* 1999; **2**:12–27.

66 Shapiro AMJ, Lakey JRT, Ryan EA, Korbutt GS, Toth E, Warnock GL, *et al.* Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; **343**:230–238.

67 Korc M. Normal function of the endocrine pancreas. In: Go VLW, Dimagno EP, Gardner JD, Lebenthal E, Reber HA, Scheele GA, eds. *The Pancreas*, 2nd edn. NewYork: Raven Press, 1993: 751–758.

68 Volk BW, Wellmann KF. Quantitative studies of the islets of nondiabetic patients. In: Volk BW, Arquilla ER, eds. *The Diabetic Pancreas*, 2nd edn. New York: Plenum Medical Book Co.; 1985: 117–125.

69 Lakey JRT, Warnock GL, Rajotte RV, Suarez-Alamazor ME, Ao Z, Shapiro AM, *et al.* Variables in organ donors that affect the recovery of human islets of Langerhans. *Transplantation* 1996; **61**:1047–1053.

70 Lakey JRT, Rajotte RV, Warnock GL, Kneteman NM. Human pancreas preservation prior to islet isolation. *Transplantation* 1995; **59**:689–694.

71 Kneteman NM, Warnock GL, Evans MG, Dawidson I, Rajotte RV. Islet isolation from human pancreas stored in UW solution for 6 to 26 hours. *Transplant Proc* 1990; **22**:763–764.

72 Lakey JRT, Warnock GL, Shapiro AMJ, Korbutt GS, Ao Z, Kneteman NM, *et al*. Intraductal collagenase delivery into the human pancreas using syringe loading or controlled perfusion. *Cell Transplant* 1999; **8**:285–292.

73 Johnson PR, White SA, London NJ. Collagenase and human islet isolation. *Cell Transplant* 1996; **5**:437–452.

74 Linetsky E, Bottino R, Lehmann R, Alejandro R, Inverardi L, Ricordi C. Improved human islet isolation using a new enzyme blend, liberase. *Diabetes* 1997; **46**:1120–1123.

75 Ricordi C, Lacy PE, Scharp DW. Automated islet isolation from human pancreas. *Diabetes* 1989; **38**(Suppl 1):140–142.

76 Ao Z, Lakey JRT, Rajotte RV, Warnock GL. Collagenase digestion of canine pancreas by gentle automated dissociation in combination with ductal perfusion optimizes mass recovery of islets. *Transplant Proc* 1992; **6**:2787.

77 Walsh TJ, Eggleston JC, Cameron JL. Portal hypertension, hepatic infarction, and liver failure complication pancreatic islet autotransplantation. *Surgery* 1982; **91**:485–487.

78 Gores PF, Sutherland DER. Pancreatic islet transplantation: is purification necessary? *Am J Surgery* 1993; **166**:538–542.

79 Oberholzer J, Triponez F, Mage R, Andereggen E, Bühler L, Crétin N, *et al*. Human islet transplantation: lesson from 13 autologous and 13 allogenic transplatations. *Transplantation* 2000; **6**:1115–1123.

80 Kahan BD. Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. *Lancet* 2000; **356**:194–202.

81 Kneteman NM, Lakey JRT, Wagner T, Finegood D. The metabolic impact of rapamycin (Sirolimus) in chronic canine islet graft recipients. *Transplantation* 1996; **61**:1206–1210.

82 Panz VR, Bonegio R, Raal FJ, Maher H, Hsu HC, Joffe BI. Diabetogenic effect of tacrolimus in South African patients undergoing kidney transplantation. *Transplantation* 2002; **73**:587–590.

83 Gruessner RW, for the Tacrolimus Pancreas Transplant Study Group. Tacrolimus in pancreas transplantation: a multicenter analysis. *Clin Transplant* 1997; **11**:299–312.

84 Maes BD, Kuypers D, Messiaen T, Evenepoel P, Mathieu C, Coosemans W, *et al.* Posttransplantation diabetes mellitus in FK-506- treated renal transplant recipients: analysis of incidence and risk factors. *Transplantation* 2001; **72**:1655–1661.

85 Tamura K, Fujimura T, Tsutsumi T, Yamamoto T, Nakamura K, Koibuchi Y, *et al.* Transcriptional inhibition of insulin by FK506 and possible involvement of FK506 binding protein-12 in pancreatic β-cell. *Transplantation* 1995; **59**:1606–1613.

86 Ishizuka J, Gugliuzza KK, Wassmuth Z, Hsieh J, Sato K, Tsuchiya T, *et al.* Effects of FK506 and cyclosporine on dynamic insulin secretion from isolated dog pancreatic islets. *Transplantation* 1993; **56**:1486–1490.

87 Cosio FG, Pesavento TE, Osei K, Henry ML, Ferguson RM. Post-transplant diabetes mellitus: increasing incidence in renal allograft recipients transplanted in recent years. *Kidney Int* 2001; **59**: 732–737.

88 Nielsen JH, Mandrup-Poulsen T, Nerup J. Direct effects of cyclosporin A on human pancreatic β-cells. *Diabetes* 1986; **35**:1049–1052.

89 Lakey JR, Rajotte RV, Warnock Gl, Kneteman NM. Pancreas procurement and preservation: impact on islet recovery and viability. *Transplantation* 1995; **59**:689–694.

90 Tsujimura T, Kuroda Y, Kin T, Avila JG, Rajotte RV, Korbutt GS, *et al*. Human islet transplantation from pancreases with prolonged cold ischemia using additional preservation by the two-layer (UW solution/perflurochemical) cold storage method. *Transplantation* 2002; **74**:1687–1691.

91 Ramachandran S, Desai NM, Goers TA, Benshof N, Olack B, Shenoy S, *et al*. Improved islet yields from pancreas preserved in perfluorocarbon is via inhibition of apoptosis mediated by mitochondrial pathway. *Am J Transplant* 2006; **6**:1696–1703.

92 Ricordi C, Fraker C, Szust J, Al-Abdullah I, Poggioli R, Kirlew T, *et al*. Improved human islet isolation outcome from marginal donors following addition of oxygenated perfluorocarbon to the cold-storage solution. *Transplantation* 2003; **75**:1524–1527.

93 Kin T, Mirbolooki M, Salehi P, Tsukada M, O'Gorman D, Imes S, *et al*. Islet isolation and transplantation outcomes of pancreas preserved with University of Wisconsin solution versus two-layer method using preoxygenated perfluorocarbon. *Transplantation* 2006; **82**:1286–1290.

94 Hering BJ, Kandaswamy R, Harmon JV, Ansite JD, Clemmings SM, Sakai T, *et al*. Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. *Am J Transplant* 2004; **4**:390–401.

95 Ichii H, Sakuma Y, Pileggi A, Fraker C, Alvarez A, Montelongo J, *et al*. Shipment of human islets for transplantation. *Am J Transplant* 2007; **7**:1010–1020.

96 Sabek OM, Cowan P, Fraga DW, Gaber AO. The effect of isolation methods and the use of different enzymes on islet yield and *in vivo* function. *Cell Transplant* 2008; **17**:785–792.

97 Weimar B, Rauber K, Brendel MD, Bretzel RG, Rau WS. Percutaneous transhepatic catheterization of the portal vein: a combined CT- and fluoroscopy-guide technique. *Cardiovasc Intervent Radiol* 1999; **22**:342–344.

98 Baidal DA, Froud T, Ferreira JV, Khan A, Alejandro R, Ricordi C. The bag method for islet cell infusion. *Cell Transplant* 2003; **12**:809–813.

99 Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, *et al.* Five-year follow-up after clinical islet transplantation. *Diabetes* 2005; **54**:2060–2069.

100 Shapiro AMJ, Ryan EA, Lakey JR. Islet transplantation in the treatment of diabetes. In: Barnett AH, ed. *Best Practice and Research Compendium*, Elsevier, 2005.

101 Hering BJ, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, *et al.* Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA* 2005; **293**:830–835.

102 Shapiro AMJ, Koh A, Salam A, *et al.* Impact of different induction therapies on long-term durability of insulin independence after clinical islet transplantation. *Am J Transplant* 2008; Suppl. A212.

103 Shapiro AMJ, Koh A, Kin T, *et al.* Outcomes following alemtuzumab induction in clinical islet transplantation. *Am J Transplant* 2008; Suppl. A213.

104 Koh A, Imes S, Ryan E, Shapiro AMJ, Senior P. Improved tolerability of tacrolimus plus mycophenolate mofetil without graft compromise in islet transplantation. *Diabetes* 2008: **57**(Suppl 1):A541.

105 Berney T, Secchi A. Rapamycin in islet transplantation: friend or foe? *Transpl Int* 2009; **22**:153–161.

106 Ryan EA, Shandro T, Green K, Paty BW, Senior PA, Bigam D, *et al.* Assessment of the severity of hypoglycaemia and glycaemic lability in type 1 diabetic subjects undergoing islet transplantation. *Diabetes* 2004; **53**:955–962.

107 Clarke WL, Cox DJ, Gonder-Frederick LA, Julian D, Schlundt D, Polonsky W. Reduced awareness of hypoglycemia in adults with IDDM: a prospective study of hypoglycemic frequency and associated symptoms. *Diabetes Care* 1995; **18**:517–522.

108 Diem P, Redmon JB, Abid M, Moran A, Sutherland DE, Halter JB, *et al.* Glucagon, cathecholamine and pancreatic polypeptide secretion in type 1 diabetic recipients of pancreas allografts. *J Clin Invest* 1990; **86**:2008–2013.

109 Paty BW, Lanz K, Kendall DM, Sutherland DE, Robertson RP. Restored hypoglycemic counterregulation is stable in successful pancreas transplant recipients for up to 19 years after transplantation. *Transplantation* 2001; **72**:1103–1107.

110 Kendall DM, Rooney DP, Smets YFC, Bolding LS, Robertson RP. Pancreas transplantation restores epinephrine response and symptom recognition during hypoglycaemia in patients with long-standing type 1 diabetes and autonomic neuropathy. *Diabetes* 1997; **46**:249–257.

111 Paty BW, Ryan EA, Shapiro AMJ, Lakey JR, Robertson RP. Intrahepatic islet transplantation in type 1 diabetic patients does not restore hypoglycaemic hormonal counterregulation or symptom recognition after insulin independence. *Diabetes* 2002; **51**: 3428–3434.

112 Meyer C, Hering BJ, Grossmann R, Brandhorst H, Brandhorst D, Gerich J, *et al.* Improved glucose counterregulation and autonomic symptoms after intraportal islet transplants alone in patients with long-standing type 1 diabetes mellitus. *Transplantation* 1998; **66**: 233–240.

113 Rickels MR, Schutta MH, Mueller R, Markmann JF, Barker CF, Naji A, *et al.* Islet cell hormonal responses to hypoglycaemia after human islet transplantation for type 1 diabetes. *Diabetes* 2005; **54**:3205–3211.

114 Rickels MR, Schutta MH, Mueller R, Kapoor S, Markmann JF, Naji A, *et al.* Glycemic thresholds for activation of counterregulatory hormone and symptom responses in islet transplant recipients. *J Clin Endocrinol Metab* 2007; **92**:873–879.

115 Lee TC, Barshes NR, O'Mahony CA, Nguyen L, Brunicardi FC, Ricordi C, *et al.* The effect of pancreatic islet transplantation on progression of diabetic retinopathy and neuropathy. *Transplant Proc* 2005; **37**:2263–2265.

116 Thompson DM, Begg IS, Harris C, Ao Z, Fung MA, Meloche RM, *et al.* Reduced progression of diabetic retinopathy after islet cell transplantation compared with intensive medical therapy. *Transplantation* 2008; **85**:1400–1405.

117 Senior PA, Zeman M, Paty BW, Ryan EA, Shapiro AMJ. Changes in renal function after clinical islet transplantation: four-year observational study. *Am J Transplant* 2007; **7**:91–98.

118 Hoving P, Rossing P, Tarnow L, Parving HH. Smoking and progression of diabetic nephropathy in type 1 diabetes. *Diabetes Care* 2003; **26**:911–916.

119 Bjorck S, Nyberg G, Mulec H, Granerus G, Herlitz H, Aureil M. Beneficial effects of angiotensin converting enzyme inhibition on renal function in patients with diabetic nephropathy. *Br Med J* 1986; **293**:471–474.

120 Fung MA, Warnock GL, Ao Z, Keown P, Meloche M, Shapiro RJ, *et al.* The effect of medical therapy and islet cell transplantation on diabetic nephropathy: an interim report. *Transplantation* 2007; **84**:17–22.

121 Albaker W, Koh A, Ryan EA, Shapiro AM, Senior P. Diabetic peripheral neuropathy is stabilized after clinical islet transplantation: 7 year follow up study. *Endo* 2008; OR26-2.

122 Koh A, Ryan E, Welsh R, Shuaib A, Shapiro AMJ, Senior P. Cardiovascular disease remains stable after islet transplantation. *Diabetes* 2008; **57**(Suppl 1):A108.

123 Rafael E, Ryan EA, Paty BW, Oberholzer J, Imes S, Senior P, *et al.* Changes in liver enzymes after clinical islet transplantation. *Transplantation* 2003; **76**:1280–1284.

124 Bhargava R, Senior PA, Ackerman TE, Ryan EA, Paty BW, Lakey JR, *et al.* Prevalence of hepatic steatosis after islet transplantation and its relation to graft function. *Diabetes* 2004; **53**:1311–1317.

125 Cure P, Pileggi A, Froud T, Norris PM, Baidal DA, Cornejo A, *et al.* Alterations of the female reproductive system in recipients of islet grafts. *Transplantation* 2004; **78**:1576–1581.

126 Alfadhli E, Koh A, Albaker W, Bhargava R, Ackerman T, McDonald C, *et al.* High prevalence of ovarian cysts in pre-menopausal women receiving sirolimus and tacrolimus after clinical islet transplantation. *Transplant Int* 2009; **22**:622–625.

127 Ryan EA, Lakey JR, Paty BW, Imes S, Korbutt GS, Kneteman NM, *et al.* Successful islet transplantation: continued insulin reserve provides long-term glycaemic control. *Diabetes* 2002; **51**:2148–2157.

128 Senior PA, Paty BW, Cockfield SM, Ryan EA, Shapiro AMJ. Proteinuria developing after clinical islet transplant resolves with sirolimus withdrawal and increased tacrolimus dosing. *Am J Transplant* 2005; **5**:2318–2323.

129 van Gelder T, Hilbrands LB, Vanrenterghem Y, Weimar W, de Fijter JW, Squifflet JP, *et al.* A randomized double-blind, multicenter plasma concentration controlled study of the safety and efficacy of oral mycophenolate mofetil for the prevention of acute rejection after kidney transplantation. *Transplantation* 1999; **68**: 261–266.

130 Campbell PM, Salam A, Ryan EA, Senior P, Paty BW, Bigam D, *et al.* Pretransplant HLA antibodies are associated with reduced graft survival after clinical islet transplantation. *Am J Transplant* 2007; **7**:1242–1248.

131 Campbell PM, Senior PA, Salam A, Labranche K, Bigam DL, Kneteman NM, *et al.* High risk of sensitization after failed islet transplantation. *Am J Transplant* 2007; **7**:2311–2317.
132 Rood PPM, Cooper DKC. Islet xenotransplantation: are we really ready for clinical trials? *Am J Transplant* 2006; **6**:1269–1274.
133 Gangaram-Panday ST, Faas MM, de Vos P. Towards stem-cell therapy in the endocrine pancreas. *Trends Mol Med* 2007; **13**:164–173.
134 Bonner-Weir S, Weir GC. New sources of pancreatic β-cells. *Nat Biotech* 2005; **23**:857–861.
135 Davalli AM, Scaglia L, Zangen DH, Hollister J, Bonner-Weir S, Weir GC. Vulnerability of islets in the immediate posttransplantation period: dynamic changes in structure and function. *Diabetes* 1996; **45**:1161–1167.
136 Bell E, Cao X, Moibi H, Greene SR, Young R, Trucco M, *et al.* Rapamycin has a deleterious effect on MIN-6 cells and rat and human islets. *Diabetes* 2003; **52**:2731–2739.
137 Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Källen R, *et al.* Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet* 2002; **360**:2039–2045.
138 Rickels MR, Schutta MH, Markmann JF, Barker CF, Naji A, Teff KL. β-Cell function following human islet transplantation for type 1 diabetes. *Diabetes* 2005; **54**:100–106.
139 Narang AS, Sabek O, Gaber AO, Mahato RI. Co-expression of vascular endothelial growth factor and interleukin-1 receptor antagonist improves human islet survival and function. *Pharm Res* 2006; **23**:1970–1982.
140 Contreras JL, Eckstein C, Smyth CA, Bilbao G, Vilatoba M, Ringland SE, *et al.* Activated protein C preserves functional islet mass after intraportal transplantation: a novel link between endothelial cell activation, thrombosis, inflammation and islet cell death. *Diabetes* 2004; **53**:2804–2814.
141 Emamaullee JA, Stanton L, Schur C, Shapiro AMJ. Caspase inhibitor therapy enhances marginal mass islet graft survival and preserves long term function is islet transplantation. *Diabetes* 2007; **56**:1289–1298.
142 Johansson H, Goto M, Dufrane D, Siegbahn A, Elgue G, Gianello P, *et al.* Low molecular weight dextran sulfate: a strong candidate drug to block IBMIR in clinical islet transplantation. *Am J Transplant* 2006; **6**:305–312.
143 Cabric S, Sanchez J, Lundgren T, Foss A, Felldin M, Källen R, *et al.* Islet surface heparinization prevents the instant blood-mediated inflammatory reaction in islet transplantation. *Diabetes* 2007; **56**:2008–2015.
144 Faradji RN, Monroy K, Riefkohl A, Lozano L, Gorn L, Froud T, *et al.* Continuous glucose monitoring system for early detection of graft dysfunction in allogenic islet transplant recipients. *Transplant Proc* 2006; **38**:3274–3276.
145 Pileggi A, Ricordi C, Alessiani M, Inverardi L. Factors influencing islets of Langerhans graft function and monitoring. *Clinica Chim Acta* 2001; **310**:3–16.
146 Paty BW, Bonner-Weir S, Laughlin MR, McEwan AH, Shapiro AMJ. Toward development of imaging modalities for islets after transplantation: insights from the National Institutes of Health Workshop on beta cell imaging. *Transplantation* 2004; **77**:1133–1137.
147 Ghofaili KA, Fung M, Ao Z, Meloche M, Shapiro RJ, Warnock GL, *et al.* Effect of exenatide on beta cell function after islet transplantation in type 1 diabetes. *Transplantation* 2007; **83**:24–28.
148 Smith RN, Kent SC, Nagle J, Selig M, Iafrate AJ, Najafian N, *et al.* Pathology of an islet transplant 2 years after transplantation: evidence for a nonimmunological loss. *Transplantation* 2008; **86**:54–62.
149 Adams AB, Shirasugi N, Jones TR, Durham MM, Strobert EA, Cowan S, *et al.* Development of a chimeric anti-CD40 monoclonal antibody that synergizes with LEA29Y to prolong islet allograft survival. *J Immunol* 2005; **174**:542–550.
150 Vincenti F, Larsen C, Durrbach A, Wekerle T, Nashan B, Blancho G, *et al.* Belatacept Study Group. Costimulation blockade with belatacept in renal transplantation. *N Engl J Med* 2005; **353**:770.
151 Beck J, Angus R, Madsen B, Britt D, Vernon B, Nguyen KT. Islet encapsulation: strategies to enhance islet cell functions. *Tissue Eng* 2007; **13**:589–599.

Gene therapy

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Keypoints

- Gene therapy for diabetes can be defined as transfer of DNA to somatic cells in order to understand, treat or prevent the disease or its complications.
- Because the etiology of diabetes is multifactorial in most cases, gene therapy aimed at correcting a single missing or multifunctioning gene will probably not be meaningful. Instead, gene therapy will need to interfere with more distal steps in the pathogenesis of diabetes, correct insulin deficiency or treat secondary complications.
- It is possible to outline several different gene transfer strategies for diabetes; prevention of β-cell destruction could be achieved by manipulating β-cells to produce a protection or survival factor.
- β-Cell destruction might also be prevented by immunomodulation.
- Stimulation of β-cell differentiation and regeneration might involve gene therapy with transcription factors that control β-cell development.
- Ectopic production of insulin by substitute cells has been achieved with fibroblasts, hepatocytes, myocytes, pituitary and exocrine cells.
- Although experimental results are promising, none of the proposed gene therapy models for treatment or prevention of diabetes have reached the stage of clinical testing.
- Clinical trials are in progress with a view to treat diabetic complications such as foot ulcer and limb ischemia.

Gene transfer techniques for genetic modification of pancreatic islets

The ability to engineer pancreatic β-cells is a prerequisite for a successful application of most gene therapy approaches in diabetes. Because pancreatic islets are terminally differentiated cell clusters, gene transfer into islet cells poses significant technical hurdles. To date, several gene therapy vectors have demonstrated their utility in genetic modification of islet cells (Table 61.3). While viral vectors, such as adenovirus [1], lentivirus [2], retrovirus [3] and adeno-associated virus [4], show the most promising gene transfer efficiency into islet cells, it is likely that non-viral vector systems will more easily satisfy biosafety concerns in clinical trials.

Table 61.3 Properties of gene therapy vectors with demonstrated utility in islet transduction.

Delivery method		Advantages	Disadvantages
Non-viral vectors	Naked DNA DNA complexes Peptide transduction domains	High clinical safety Easy and inexpensive to produce Non-immunogenic Unlimited capacity	Low transfection efficiency Transient gene expression
Adenovirus		High transduction efficiency High viral titer Infects non-dividing cells	Transient gene expression Immunogenic Clinical safety issues
Adeno-associated virus		Potential site-specific integration Infects non-dividing cells No immune response High clinical safety	Low capacity (5 kb) Very difficult to generate
Lentivirus		High transduction efficiency Long term expression Easy to generate Relatively high titer	Possibility of insertional mutagenesis with clinical effects
Herpes simplex virus		High transduction efficiency Up to 30 kb insertion	Inflammatory and toxic reactions in patients Complicated genome and propagation

Prevention of β-cell destruction in type 1 diabetes

β-Cell survival factors

Prevention of β-cell destruction could be achieved by genetic manipulation of β-cells so that they produce a β-cell protection and/or survival factor. Such an approach would, in general, leave the immune system unaffected as the transgene production is localized to the islets. Targeting of a survival factor to the β-cells could be applied to individuals in whom autoimmune destruction of β-cells has begun, but not reached the end stage. Candidate transgenes currently under investigation include those encoding antioxidant enzymes: glutathione peroxidase, mitochondrial manganese superoxide dismutase (MnSOD), catalase, cytosolic copper-zinc superoxide dismutase, anti-apoptotic proteins (members of the heat shock proteins and Bcl-2 families) and modulators of cytokine signaling pathways (modulation of nuclear factor κB, NFκB), and suppressors of cytokine signaling. Alternative approaches for cytoprotection of β-cells have been envisaged, such as immune modulators; for example, an interleukin-1 (IL-1) receptor antagonist, hepatocyte growth factor, transforming growth factor β (TGF-β), adenoviral E3 and calcitonin gene-related peptide, inhibitors of Fas ligand signaling, and antistress factors such as thioredoxin. These factors have been addressed experimentally and could possibly, when expressed by β-cells in patients with diabetes, promote β-cell survival.

Genetic modulation of the immune system

In individuals with a high risk of developing T1DM, as indicated by genetic and humoral markers, but who have not yet entered the phase of autoimmune β-cell destruction, it might be possible to prevent the progression of the disease by DNA vaccination. In mice, it has already been observed that DNA vaccination with a glutamic acid decarboxylase 65 (GAD65) gene construct generates a protective humoral immune response and significantly delays the onset of diabetes [5]. Other studies of DNA vaccination in non-obese diabetic (NOD) mice were performed with a preproinsulin/glutamic acid decarboxylase 65 (Ins-GAD) fusion construct as the target antigen to introduce a larger number of autoantigenic target epitopes. DNA co-vaccination with Ins-GAD and B7-1wa (a membrane-bound molecule that can engage cytotoxic T-lymphocyte associated antigen 4 [CTLA-4] and promote negative signaling) generated protective regulatory T-cells and ameliorated the disease [6]. Both insulin and GAD65 may be key autoantigens in T1DM, and if the DNA vaccination approach leads to tolerance, β-cell destruction might be avoided.

Furthermore, virus-based IL-10 genetic studies showed beneficial effects on the development of T1DM, both effectively blocking progression of insulitis as well as suppressing autoimmunity in NOD mice transplanted with syngeneic islet grafts [7]. Despite these results, significant immunosuppression, resulting from systemic expression of high levels of IL-10, give rise to serious concerns regarding the applicability of such an approach in clinical settings; however, the development of viral vectors promoting a tightly regulated transgene expression could overcome this obstacle.

Targeted immunomodulation has also been achieved by transducing autoantigen-specific $CD4^+$ T-cells *ex vivo* with retrovi-

ruses that encode immune regulatory proteins [8]. Following intravenous injection of the transduced T-cells, it appears that the cells accumulate at the site of inflammation. Using this site-specific delivery, immune regulatory proteins have been observed to protect against autoimmune reactions, possibly by converting the immune reaction from a Th1 to a Th2 response.

An alternative approach may be targeting expression of disease-specific epitopes to activated B lymphocytes [9]. Antigen presentation by these cells seems to result in immunosupression and therapeutic efficacy. It is not clear why this is the case, but it has been suggested that antigen presentation by B lymphocytes leads to immune downregulation, whereas presentation by macrophages or dendritic cells is immune stimulatory.

Stimulation of β-cell differentiation and regeneration

A better understanding of the cellular sources for the expansion and turnover of β-cells seen in postnatal life could make way for a possible gene therapy approach leading to *in situ* regeneration of β-cells in patients with diabetes. Indirect evidence has suggested that postnatal β-cells derive from adult stem cells, proposed to reside in the pancreatic ducts, bone marrow, spleen or within islets. Lineage tracing experiments, however, demonstrated that the vast majority of adult β-cells derive from preexisting β-cells, suggesting that terminally differentiated β-cells retain a significant proliferative capacity and thus could represent an attractive target for expansion using gene therapy [10]. For example, mice over-expressing gastrin and TGF-α display a significantly increased islet cell mass. In addition, the cyclin-dependent kinase 4 (CDK4) and cyclins D1/D2 emerge as key determinants of β-cell proliferation and β-cell mass. Indeed, mice lacking CDK4 display a selective loss of β-cell function and over-expression of CDK4 results in preferential hyperplasia of the β-cells; however, it is likely that all approaches aiming at regenerating β-cells in patients with T1DM must be combined with a strategy to prevent autoimmune destruction of the newly formed β-cells.

Ectopic production of insulin by substitute cells

With the cloning of the insulin gene in the late 1970s, it was proposed that using a suitable promoter and insulin gene construct, non-insulin-producing cells could be made into insulin-producing substitute cells, and that such cells could restore insulin production in T1DM and some patients with T2DM. The original experiments were carried out on cell lines and fibroblasts, but since then hepatocytes, myocytes, pituitary and exocrine cells have also been made into insulin-producing substitute cells. Proinsulin is only converted into insulin by the prohormone convertases PC2 and PC3, which are not expressed in most substitute cells. This limitation has been overcome by mutating the insulin gene so that a furin cleavable site is generated (INS-FUR). The protease furin, unlike PC2 and PC3, is expressed in most cells. Although processing of proinsulin to insulin can be achieved in substitute cells, none of these cells would be able to respond to insulin secretagogues with a physiologic secretion of insulin. Recent studies showed that both lentivirus [11] and adeno-associated virus vectors [12] were able to deliver INS-FUR cDNA efficiently to hepatocytes *in vitro* and *in vivo*, resulting in long-term correction of diabetes in rats.

Given its role in β-cell maintenance, the transcription factor Pdx-1 was investigated for its ability to induce ectopic insulin production in non β-cells *in vivo*. Ferber and colleagues have used adenoviral-mediated Pdx-1 overexpression for *ex vivo* transduction of adult human hepatocytes for cell-based therapy. In addition to glucagons, insulin and somatostatin, exogenous expression of Pdx-1 induced transcription of several β-cell products, including glucose transporter 2 and glucokinase, endogenous Pdx-1 and multiple downstream pro-endocrine developmental factors [13].

In a recent study Zhou *et al*. describe a way to reprogram pancreatic exocrine cells into insulin producing β-cells [14]. By using adenoviruses, they introduced combinations of nine different genes, previously identified to be essential to the embryonic development of β-cells, into the pancreas of live mice. They found that the transfer of three transcription factors (Ngn3, Pdx1 and Mafa) induces trans-differentiation of up to 20% of the successfully manipulated exocrine cells into β-like cells. By lineage tracing experiments, the authors proved that the trans-differentiated cells were, indeed, exocrine cells. These reprogrammed β-cells had similar morphology, protein expression and the capacity to secrete insulin as their "natural" counterparts. Moreover, they were able to improve hyperglycemia in mice with induced diabetes. This study elegantly shows that adult cells can be directly converted into another type of adult cell, which opens the possibility of directly converting cells *in vivo* for repair and regeneration as a therapeutic tool for diabetes and beyond [14].

Cell therapy using β-cells derived from embryonic stem cells

This topic is discussed in detail in the first part of this chapter.

Ex vivo gene transfer to islets destined for transplantation

Pancreatic islet transplantation has been validated as a realistic alternative to correct the insulin deficiency in T1DM; however, islets are exposed to a plethora of insults before, during, after isolation and at the site of implantation, all of which could result in cellular death and impaired function, therefore reducing the yield of viable islets that engraft after implant. As a result, much effort has been made to understand better the molecular mechanisms involved in all these processes as well as in developing new therapeutic strategies to increase durable functional islet mass. In

Table 61.4 Examples of gene therapy-based strategies leading to improved outcome of islet transplantation in animal models.

Gene product	Delivery method	Animal model	Desired effect
HGF and IL-1Ra	Adenovirus	STZ-induced-diabetic NOD-SCID mice	β-cell survival, β-cell proliferation, increased revascularization
HGF	Adenovirus	Allogeneic transplantation rat model	β-cell survival, β-cell proliferation
IL-10	Adenovirus AAV	STZ-induced diabetic rats (allotransplant) Autoimmune recurrence	Immunotolerance
sCD40-Ig	Adenovirus	Allogeneic transplantation mouse model	Immunotolerance
XIAP	Adenovirus	Chemically diabetic immunodeficient mice STZ-induced diabetic mice (allotransplant)	β-cell survival
Akt	Adenovirus	STZ-induced diabetic SCID mice	β-cell survival
IRAP	Adenovirus	STZ-induced diabetic rats	β-cell survival
TNFR-Ig	Adenovirus	Diabetic mice (allotransplant)	Immunotolerance
VEGF	Non-viral	Diabetic mice	Increased revascularization
MnSOD	Adenovirus	STZ-induced diabetic NODscid mice	β-cell protection against oxidative damage
TGF-β1	Adenovirus	Autoimmune recurrence in NOD mice	β-cell survival
Bcl2	Adenovirus Adenovirus	Xenotransplantation Diabetic SCID mice	β-cell survival

HGF, hepatocyte growth factor; Ig, immunoglobulin; IL, interleukin; IL-1Ra, interleukin-1 receptor antagonist; IRAP, interleukin-1 receptor antagonist protein; MnSOD, manganese superoxide dismutase; NOD, non-obese diabetic; SCID, severe combined immunodeficiency; STZ, streptozotocin; TGF, transforming growth factor; TNFR, tumor necrosis factor receptor; VEGF, vascular endothelial growth factor; XIAP, X-linked inhibitor of apoptosis.

this context, *ex vivo* gene transfer to pancreatic islets represents an attractive approach towards enhancing graft survival after transplantation. Most of the gene therapy strategies described above show promise for the cytoprotection of islets in transplant settings and may ultimately promote significantly enhanced function and survival of transplanted islets leading to an improved outcome of the transplantation procedures (Table 61.4); however, considering the complex pathways involved in β-cell destruction and loss of function following islet transplantation, it is likely that gene therapy strategies targeting multiple genes, using multicistronic vectors, will be more beneficial for the improvement of graft survival. For this purpose, a number of factors still remain to be investigated to find which combination of genes proves optimal, what threshold level of gene expression is required and what delivery strategy is the most efficient for targeting the mechanisms leading to graft failure.

Gene therapy strategies for type 2 diabetes

T2DM is characterized by hyperglycemia combined with variable degrees of insulin resistance. A potential therapeutic approach for the treatment of T2DM is the use of GLP-1, a gut-derived incretin hormone which has important roles in glucose homeostasis. Using an adenoviral vector to express GLP-1 with the insulin leader sequence to ensure secretion, Lee *et al.* [15] have recently demonstrated transient improvement in glucose tolerance using an overt T2DM animal model. Significant therapeutic effects, including improved glucose homeostasis, decreased weight gain, reduced hepatic fat and improved adipokine profile, were also obtained by constitutively expressing exendin-4, a GLP-1 receptor (GLP-1R) agonist in a high fat induced obesity mouse model [16]). These studies suggest that GLP-1/GLP-1R agonist gene therapy may be a promising treatment modality for T2DM.

Another interesting line of research exploits the potential of viral proteins as therapeutic tools for treating glycemic dysregulation in humans. Human adenovirus type 36 (Ad-36) was determined as a novel candidate for improving metabolic profile by expanding adipose tissue while enhancing insulin sensitivity in experimentally infected rats [17]. Recent studies have demonstrated the capacity of Ad-36 to increase glucose uptake by adipose tissue explants obtained from subjects with and without diabetes. Ad-36 upregulated expression of several pro-adipogenic genes, adiponectin and fatty acid synthetase, and reduced the expression of inflammatory cytokine macrophage chemoattractant protein-1 in a phosphotidylinositol 3-kinase dependent manner [18]. Moreover, Ad-36 was also able to enhance glucose uptake in primary skeletal muscle cells from healthy lean subjects and subjects with diabetes [19]. Therefore, the potential of viral proteins to enhance glucose disposal and improve adipose and skeletal muscle tissues metabolic profile could be used as therapeutic targets for humans.

Gene therapy for the treatment of diabetic complications

The gene therapy models for treatment or prevention of T1DM and T2DM outlined above have not yet reached clinical stages. By contrast, the use of gene therapy in the treatment of diabetes complications shows great promise and some projects are in the early stages of clinical testing. Briefly, transduction of nerves by herpes virus expressing nerve growth factor has been investigated for treatment of cystopathy and for peripheral neuropathy. In addition, the effects of gene therapy with vascular endothelial growth factor (VEGF) on coronary artery disease have been studied in a clinical trial. It has been shown that VEGF is required to initiate immature vascular formation, whereas angiopoietin-1 (Ang-1) is essential for the maintenance of endothelial integrity and vascular formation. In this regard, a recent study demonstrated that Ang-1 gene therapy promotes vascular maturation and stabilization and rescues diabetes impaired myocardial angiogenesis and myocardial remodeling in *db/db* mice [20]. Another small clinical trial showed significant improvement in patients with diabetes and diffuse peripheral vascular disease after intramuscular administration of a VEGF gene-carrying plasmid. Furthermore, delivery of insulin-like growth factor I by non-viral gene therapy in combination with autologous cell transplantation improved diabetic wound healing significantly in a preclinical large-animal model [21].

Problems and future directions

Although significant progress is currently being made in the field of gene therapy of diabetes, it should be emphasized that gene therapy is neither a low cost:benefit nor a low risk:benefit approach. For example, there is the obvious risk that the gene therapy could induce severe hypoglycemia if the number of gene modified insulin-producing cells and their insulin release is not under perfect control. In addition, we need to await ongoing developments in gene transfer techniques to achieve therapeutic long-term expression, *in vivo* regulation of transgene expression and lack of immune triggering, in order to conduct gene therapy on β-cells *in vivo*. Because T1DM is usually more severe than T2DM, it is natural to assume that the first gene therapy efforts will be dedicated to the treatment of T1DM, and that the experiences from these trials will be used for later strategies for the treatment of T2DM. Finally, it must be clear that the benefits of gene therapy of diabetes outweigh the risks and offer advantages compared to those of conventional treatment before this approach could become accepted in general practice.

References

1 Gonçalves MA, de Vries AA. Adenovirus: from foe to friend. *Rev Med Virol* 2006; **16**:167–186.

2 Cockrell AS, Kafri T. Gene delivery by lentivirus vectors. *Mol Biotechnol* 2007; **36**:184–204.

3 Dalba C, Bellier B, Kasahara N, Klatzmann D. Replication-competent vectors and empty virus-like particles: new retroviral vector designs for cancer gene therapy or vaccines. *Mol Ther* 2007; **15**:457–466.

4 Wu Z, Asokan A, Samulski RJ. Adeno-associated virus serotypes: vector toolkit for human gene therapy. *Mol Ther* 2006; **14**:316–327.

5 Goudy KS, Wang B, Tisch R. Gene gun-mediated DNA vaccination enhances antigen-specific immunotherapy at a late preclinical stage of type 1 diabetes in nonobese diabetic mice. *Clin Immunol* 2008; **129**:49–57.

6 Prud'homme GJ, Glinka Y, Khan AS, Dragia-Akli R. Electroporation-enhanced nonviral gene transfer for the prevention or treatment of immunological, endocrine and neoplastic diseases. *Current Gene Therapy* 2006; **6**:243–273.

7 Goudy KS, Tish R. Immunotherapy for the prevention and treatment of type 1 diabetes. *Internat Rev Immunol* 2005; **24**:307–326.

8 Fathman CG, Costa GL, Seroogy CM. Gene therapy for autoimmune disease. *Clin Immunol* 2000; **95**:S39–43.

9 Agarwal RK, Kang Y, Zambidis E, Scott DW, Chan CC, Caspi RR. Retroviral gene therapy with an immunoglobulin-antigen fusion construct protects from experimental autoimmune uveitis. *J Clin Invest* 2000; **106**:245–252.

10 Nir T, Dor Y. How to make pancreatic β cells: prospects for cell therapy in diabetes. *Curr Opin Biotechnol* 2005; **16**:524–529.

11 Ren B, O'Brien BA, Swan MA, Koina ME, Nassif N, Wei MQ, *et al.* Long-term correction of diabetes in rats after lentiviral hepatic insulin gene therapy. *Diabetologia* 2007; **50**:1910–1920.

12 Hsu PY, Kotin RM, Yang YW. Glucose- and metabolically regulated hepatic insulin gene therapy for diabetes. *Pharm Res* 2008; **25**:1460–1468.

13 Sapir T, Shternhall K, Meivar-Levy I, Bumenfeld T, Cohen H, Skutelsky E, *et al.* Cell-replacement therapy for diabetes: generating functional insulin-producing tissue from adult human liver cells. *Proc Natl Acad Sci U S A* 2005; **102**:7964–7969.

14 Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. *In vivo* reprogramming of adult pancreatic exocrine cells to β-cells. *Nature* 2008; **455**:627–632.

15 Lee Y, Kwon MK, Kang ES, Park YM, Choi SH, Ahn CW, *et al.* Adenoviral vector-mediated glucagon-like peptide 1 gene therapy improves glucose homeostasis in Zucker diabetic fatty rats. *J Gene Med* 2008; **10**:260–268.

16 Samson SL, Gonzalez EV, Yechoor V, Bajaj M, Oka K, Chan L. Gene therapy for diabetes: metabolic effects of helper-dependent adenoviral exendin 4 expression in a diet-induced obesity mouse model. *Mol Ther* 2008; **16**:1805–1812.

17 Pasarica M, Shin AC, Yu M, Ou Yang HM, Rathod M, Jen KL, *et al.* Human adenovirus 36 induces adiposity, increases insulin sensitivity, and alters hypothalamic monoamines in rats. *Obesity* 2006; **14**:1905–1911.

18 Rogers PM, Mashtalir N, Rathod MA, Dubuisson O, Wang Z, Dasuri K, *et al.* Metabolically favorable remodeling of human adipose tissue by human adenovirus type 36. *Diabetes* 2008; **57**:2321–2331.

19 Wang ZQ, Cefalu WT, Zhang XH, Yu Y, Qin J, Son L, *et al.* Human adenovirus type 36 enhances glucose uptake in diabetic and nondiabetic human skeletal muscle cells independent of insulin signaling. *Diabetes* 2008; **57**:1805–1813.

20 Chen JX, Stinnett A. Ang-1 gene therapy inhibits hypoxia-inducible factor-1α (HIF-1α)-prolyl-4-hydroxylase-2, stabilizes HIF-1α expression, and normalizes immature vasculature in db/db mice. *Diabetes* 2008; **57**:3335–3343.

21 Hirsch T, Spielmann M, Velander P, Zuhaili B, Bleiziffer O, Fossum M, *et al*. Insulin-like growth factor-1 gene therapy and cell transplantation in diabetic wounds. *J Gene Med* 2008; **10**:1247–1252.