Cell-based therapies for the management of type 1 diabetes

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Pancreatic or islet cell transplantation are well-established options for the management of type 1 diabetes. Their use in paediatric practice has been limited to islet cell auto-transplants following chronic pancreatitis. The need for ongoing immunosuppression limits the broader use of these therapeutic approaches in the management of children with type 1 diabetes. Donor supply of islets for transplantation remains the biggest limitation for the broader application of these techniques. Better, milder immunosuppression regimens coupled with alternative sources for islets and improved *in-situ* protection of the graft are options for further development. Embryonic stem cells may provide a source for beta-cells ultimately, but the development of a functioning islet remains elusive.

Type 1 diabetes is a T-cell-mediated autoimmune disease in which beta-cells are destroyed. Clinical signs arise when the autoimmune process exceeds the regenerative capacity of the beta-cells, leading to a critical reduction in cell numbers and insulin production.

Intensive insulin therapy delivered by injection or an insulin pump is the mainstay of therapy for type 1 diabetes. Tight glycaemic control to minimise the long-term micro- and macrovascular complications is the primary therapeutic goal (DCCT/EDIC [Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications] Research Group, 2009). While such interventions are effective, normalisation of HbA_{1c} is rarely achieved without the ever-present risk of hypoglycaemia, which is the main limitation to tight glycaemic control (Cryer, 2009).

Transplantation therapies for type 1 diabetes have largely centred on either solid organ transplantation or islet cell transplantation: whole pancreas transplantation is usually undertaken either alone or in combination with a kidney; and islet cell transplantation can be allogenic or an auto-transplant. Recent developments in the understanding of autoimmune diseases coupled with advances in stem cell biology have opened up new avenues for cell-based therapies in type 1 diabetes.

In this article the authors examine the current cell-based technologies. Although the introduction of these approaches to the standard clinical care of children and young people with type 1 diabetes is some way off, paediatric diabetologists need to begin to familiarise themselves with the concepts and methodologies in order to better provide future care for children and young people with type 1 diabetes.

Solid organ transplantation

The replacement of the whole pancreas has a long history, and current success rates are depicted in *Figure 1*; most recipients also receive a renal transplant for diabetic nephropathy. Graft survival of a pancreas transplant following renal transplantation is better than pancreas alone or pancreas first followed by kidney. Over an 11–20-year follow-up, HbA_{1c} was shown to normalise at a mean of 5.3% (34 mmol/mol) and diabetic nephropathy reversed after 10 years (Gruessner et al, 2008). Solid organ transplantation success rates provide a useful benchmark against which cell-based therapies can be compared.

Article points

1. Recent developments in the understanding of autoimmune diseases coupled with advances in stem cell biology have opened up new avenues for cell-based therapies in type 1 diabetes.

2. Islet cell transplantation represents a minimally invasive approach to beta-cell replacement, although the donor supply of islets remains a limitation of this technique.

3. Other islet sources are required, such as stem cells, as well as more specific immunotherapies directed towards the autoimmune process in type 1 diabetes.

Key words

– Islet cell
– Pancreas
– Stem cell
– Transplantation
– Xenograft

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Islet cell transplantation

Auto- or allogenic islet transplantation represents a minimally invasive approach to beta-cell replacement. Auto-transplantation is used in situations where pancreatic function becomes compromised or surgical removal is contemplated, such as chronic pancreatitis or benign pancreatic endocrine tumours. Post-pancreatectomy diabetes is hard to control with exogenous insulin, so transplantation is a valuable intervention.

Reported complications relate to the pancreatectomy rather than the transplant itself. The isolated islet cells are infused directly into the hepatic portal vein. The total number of islets required, 7000/kg in adults and 4000/kg in children, is less than allogenic transplantation. The islets need 3–6 months to establish in the liver, and during this time tight glycaemic control with insulin is recommended to protect the cells from glucotoxicity.

Success rates of insulin independence vary, with the large Minnesota series reporting 45% of recipients

insulin independent after 1 year, with a mean HbA$_{1c}$ of 4.6%, and a further 16% requiring only once-daily, low-dose insulin; at 3 years post-transplant, 30% remained insulin independent (Sutherland et al, 2012). The intervention is suited to children, and in the Minnesota series of 409 recipients, 53 were children aged 5–18 years. Children seemed to do better in terms of insulin independence, with 55% independent at 3 years. Islet yield correlated with the degree of pancreatic function before pancreatectomy, and also to the number of previous surgical interventions for pancreatitis.

Allogenic pancreatic islet cell transplantation for type 1 diabetes shows clear short- and long-term efficacy. This procedure has been reserved for those individuals with recurrent, severe hypoglycaemia or marked glycaemic lability, or both, as well as those already on immunosuppression after renal transplantation (NICE, 2008). Since the publication of the Edmonton experience in 2000 (Shapiro et al, 2000), a total of 677 islet-alone or islet-after-kidney transplants have been recorded in the Collaborative Islet Transplant Registry (Barton et al, 2012). Insulin independence at 3 years from transplant increased over the period 1999 to 2010, from 27% to 44%, with a concomitant reduction in HbA$_{1c}$ and resolution of severe hypoglycaemia. The increased graft survival rate and insulin independence compare favourably with the 61% insulin independence following pancreas-alone transplantation (Gruessner et al, 2008). Current insulin independence rates for islet cell transplantation are similar to those achieved with pancreas-alone transplantation in the 1990s (*Figure 1*).

Two key components of this success have been the collagenase enzymes used for islet digestion and novel protocols using T-cell depletion for induction with tumour necrosis factor-alpha inhibitors (Hering et al, 2005; Bellin et al, 2008), which has led to a reduction in the reinfusion rate to 48%. As with pancreas transplantation, use of the calcineurin inhibitor tacrolimus in low doses was not associated with the beta-cell toxicity observed at higher doses.

The cell-dosing schedule is 15 000/kg, which is about a quarter of that used in 1999 but twice that used in auto-transplants; the factors influencing islet yields and loss post-transplantation are shown in *Box 1*. Nonetheless, the numbers needed if islet cell transplantation is to become more mainstream as a
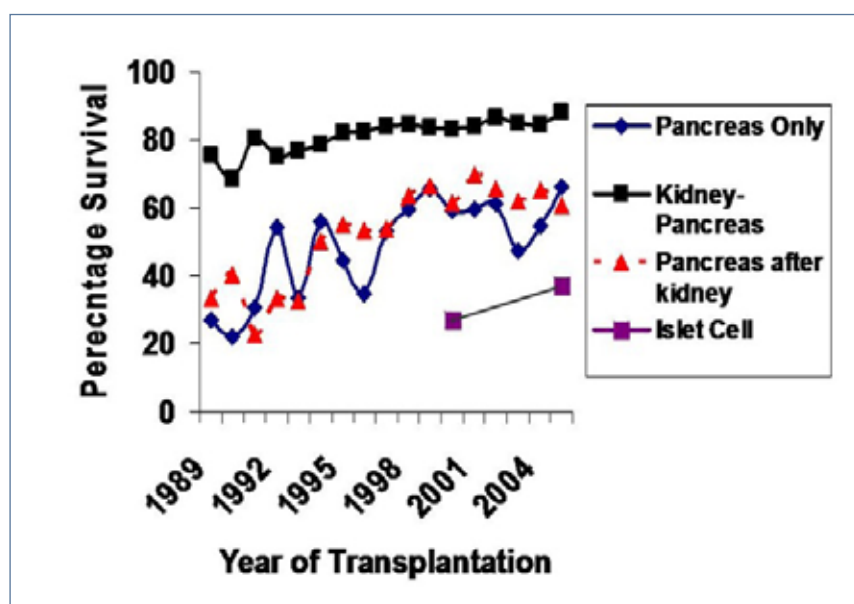

Figure 1. Unadjusted 3-year graft survival by year of transplant for solid organ and islet cell procedures. Data from Barton et al (2012) and US Department of Health and Human Services (2011).

therapy still requires a major commitment to donor recruitment as well as increasing tissue availability more specifically, and at the same time reducing allograft rejection (*Box 2*).

Islet transplantation from a live related donor after selective pancreatic resection successfully ameliorates brittle diabetes with hypoglycaemia (Matsumoto et al, 2005). However, this clearly places the donor at risk of major complications, including diabetes, in addition to the immunosuppressive side effects in the recipient.

Attempts to overcome the host's immune system by encapsulating islets in a semipermeable membrane through which nutrients and insulin can pass, but larger T- and B-cells cannot, has had limited success (Soon-Shiong et al, 1994; Elliot et al, 2007). The survival of the graft remains limited, with challenges surrounding the selection of the optimal encasing biomaterial.

It has also been suggested that alternative siting of transplanted islets may improve survival by reducing the blood-mediated inflammatory response. In cases of pancreatectomy, there may be advantages in placing autologous islets in an alternative site, such as muscle (Rafael et al, 2008).

Side effects of islet transplantation relate predominantly to the infusion procedure, particularly portal vein thrombosis or intraperitoneal bleeding. Short-term immunosuppression problems occur in up to 30% of cases (www.citregistry.org), but long-term safety data need to be acquired. There remains, however, a careful balance to be struck between establishing excellent glucose control and cessation of disabling hypoglycaemia and the long-term effects of chronic immunosuppression. Until milder immunosuppression regimens are developed and the donor recruitment issue resolved, the use of allogenic islet transplantation in children and young people will remain limited.

Strategies for increasing available cells for transplantation
Embryonic stem cells
Embryonic stem cells (ESCs) derived from the inner cell mass of blastocysts during the early stages of embryogenesis are pluripotent. As the steps of differentiation to a beta-cell are well understood (Guo and Hebrok, 2009), ESCs could provide a source for the large number of cells needed for transplantation. However, it has to be appreciated that directed differentiation of ESCs is challenging, not least because large cell-number generation has to be balanced against the formation of unwanted cell types or fates.

Initial attempts to generate beta-cells from ESCs were only partially successful as the confirmation that true conversion to a physiological beta-cell was often lacking. This is important as cells other than beta-cells can produce insulin, such as foetal hepatocytes. Also, ESC-derived beta-like cells generally secrete insulin at much lower levels and are less well regulated than true beta-cells.

Box 1. Factors influencing islet yields and post-transplantation loss.

Factors affecting yield:
- Organ procurement
- Preservation and transportation
- Isolation technique
- Culture conditions
- Apoptosis as a result of hypoxia, denervation and loss of vasculature

Factors contributing to loss of transplanted cell mass during and after transplantation:
- Immediate blood-mediated inflammatory reaction
- Recurrence of autoimmunity
- Toxicity of immunosuppressive drugs
- Allograft rejection
- Glucotoxicity
- Hepatic steatosis

Box 2. Barriers to overcome in islet cell replacement.

Reducing allograft rejection and recurrent autoimmunity:
- Better immunomodulation, e.g. alemtuzumab
- Induction of chimerism, e.g. combined islet haematopoietic stem cells
- T-regulatory cell therapy, e.g. give mismatched T-regulatory cells
- Protection of cells through encapsulation
- Bio-hybrid devices to protect cells and provide local immunomodulation

Increasing tissue availability:
- Increase number of human donors
- Improved donor selection
- Xenotransplantation
- Stimulate islet cell neogenesis from embryonic or mesenchymal stem cells
- Stimulate native islet proliferation e.g. glucagon-like peptide-1

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Directed differentiation of human ESCs has been more successful (Kroon et al, 2008). The steps mimic those observed in normal pancreas development using signals that regulate embryonic endoderm and pancreas formation. Implantation of human ESC-derived endocrine precursors results in insulin-positive cells that reduce hyperglycaemia in mouse models of diabetes, whereas removal of the cells leads to recurrence of the diabetes. These approaches have been advanced further by careful coordination in a temporal and spatial manner of the interaction of the right combination of transcription factors. For example, too early expression of the transcription factor neurogenin 3 results in dominant formation of alpha-cells to the detriment of other cell types (Johansson et al, 2007).

Despite these major advances, the cells obtained probably represent immature beta-cells with low insulin content and numerous other hormones in the same cell, as well as little response to glucose stimulation (Jiang et al, 2007). One reason for this may be that proper cell–cell interactions are not facilitated. Pancreas development is interactive with different layers secreting and responding to inductive signalling. Islets are complex three-dimensional structures consisting of different cell types and different hormones that interact closely with blood vessels and the nervous system. Co-culturing islets with mesenchymal stem cells in mice has been demonstrated to improve islet survival through direct contact or soluble mediators and is a promising new area of research (Rackham et al, 2013). Three further areas need to be explored:

- First, consideration needs to be given to the risk of tumour formation; undifferentiated ESCs associated with transplantation could lead to teratoma formation.
- Second, the ongoing immune process that causes type 1 diabetes along with the immune response to administered stem cells will probably require concomitant immunosuppression.
- Third, the pluripotential cell source needs to be considered; only four transcription factors are required to reprogramme somatic cells into induced pluripotent stem cells that can differentiate into all three germ layers (Park et al, 2008). Initial studies used viral transfection, which is problematic, but more recent studies have used small chemicals or histone deacetylase inhibitors to induce reprogramming (Huangfu et al, 2008).

Expanding endogenous beta-cell mass

It is well known that conditions such as pregnancy lead to an expansion in beta-cell mass. New beta-cells are thought to arise predominantly from existing beta-cells, with a smaller contribution from cells located in the pancreatic duct (Dor et al, 2004). The cell source probably depends upon the stimulus applied; regeneration after injury tends to be mainly from duct lining cells. Transdifferentiation of pancreatic exocrine cells or hepatic stem cells into beta-cells has been demonstrated using three transcription factors from the beta-cell differentiation pathway. Despite these advances it remains difficult *ex vivo* to expand the number of beta-cells and to maintain insulin expression over time.

Xenotransplantation

Xenotransplantation with pig islets would potentially overcome the problem of demand for islets, with an unlimited supply from colonies of isolated, disease-free herds. In addition, the use of transgenic pigs enables specific manipulation of beta-cells to ameliorate immune rejection and blood inflammatory responses (Ekser et al, 2012). Small human trials are ongoing (Elliot et al, 2007; Garkavenko et al, 2012), but rejection remains a problem.

Immunotherapies

There is increasing evidence to suggest that once the autoimmune process has been attenuated using non-diabetogenic means, beta-cell regeneration can take place even in the face of clinical manifestations of the disease (Hess et al, 2003). Several approaches have been used in humans and mice.

Autologous non-myeloablative haematopoietic stem-cell transplantation has been used to treat a variety of autoimmune conditions (Farge et al, 2010), and several groups have reported successful short-term outcomes in type 1 diabetes (Couri et al, 2009; Snarski et al, 2011). Most recipients are insulin-free immediately after transplantation, with approximately 50% insulin-free 19–31 months later (Gu et al, 2012). Hospital stays average between 18–25 days, and preconditioning requires cyclophosphamide and anti-thymocyte globulin. Alopecia, nausea and bone marrow suppression are immediate side effects. Long-term complications related to immunosuppression therapy, such as tumour formation, endocrine disorders (hypothyroidism) and infertility, remain to be defined.

Older age at onset and absence of diabetic ketoacidosis at presentation are prognostic factors for success. For the paediatric population, who tend to be younger and present more often with ketoacidosis, this approach may be less attractive, particularly when considering the risk–benefits of immunosuppression.

Current animal models offer other approaches that may obviate the need for transplantation. In non-obese diabetic mice, multiple injections of male allogenic splenocytes enables the recovery of beta-cell mass that arises from beta-cell precursors and is not related directly to the splenocyte injections (Nishio et al, 2006). This observation may translate into a more refined immunomodulation than that provided by autologous non-myeloablative haematopoietic stem-cell transplantation. Although the initial immunotherapy clinical trials are not as promising (Ludvigsson et al, 2012), autologous T-regulatory cell infusion for children and young people newly diagnosed with type 1 diabetes may be an option (Marek-Trzonkowska et al, 2012).

Conclusion

There is a clear effect of solid organ and auto-islet transplantation. Exploration of other sources, such as stem cells or capitalising on known pathways of proliferation, is required as there is likely to be a steady increase in the number of islet cell transplantations undertaken in individuals with type 1 diabetes. Any approach will have to meet high safety standards and show that the derived beta-cells secrete insulin in response to glucose in a physiological manner. This will need to be coupled with more specific immunotherapies directed towards the autoimmune process in type 1 diabetes. ■

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Page points

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