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Review

PANCREATIC ISLET TRANSPLANTATION AND REGENERATION FOR *Diabetes mellitus* TREATMENT

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The incidence of diabetes mellitus due to different causes (heredity, unhealthy food, sedentary life style, etc.) is increasing both in Latvia and worldwide every year. In almost all cases insulin therapy must be administered. However, the latest modern technologies promote the development of new and alternative treatments of diabetes mellitus. One of them, the procedure of islet transplantation is comparatively simple and relatively non-invasive in contrast to whole pancreas transplantation. It is considered as a perspective strategy in the treatment of patients with type 1 diabetes, when the insulin-producing beta cells have been destroyed and blood glucose level is above the normal range (hyperglycaemia). Successful outcome of clinical islet transplantation has been shown in several islet transplantation centres in Europe and elsewhere, particularly in the last seven years, using corticoid-free immunosuppression regimen. Islets are isolated by a collagenase-based digestion of donor pancreas with a "Ricordi" chamber, followed by a purification step in a Ficoll-based continuous density gradient. This review discusses the islet transplantation procedure and deals with the methods of islet isolation. Importance of islet preparations and engraftment quality, as well as clinical outcome and its indications also are described. The present status of islet regeneration including beta cells neogenesis and regeneration therapy strategies also are presented. The experience of Latvian scientists in islet transplantation procedure is briefly described.

Key words: pancreas, islets of Langerhans, diabetes mellitus, transplantation, regeneration.

INTRODUCTION

The pancreas contains exocrine, endocrine, and ductal cells and serves two major functions: (a) the production of digestive enzymes, such as amylase and lipase, which are secreted by exocrine acinar cells and routed to the intestine by a branched ductal network; and (b) the regulation of blood sugar, which is achieved by endocrine cells of the islets of Langerhans. The endocrine cells, arranged in islets within the pancreas, consist of alpha, beta, delta, and PP or gamma cells, which secrete peptide hormones glucagon, insulin, somatostatin, and pancreatic polypeptide (PP), respectively (Menger *et al.*, 1994).

Islets can be considered as micro-organs throughout the pancreas (Duvillie *et al.*, 2003). The islets account for about 1.3% of the volume (1–2 g in total) of the pancreas with a progressive increase in concentration from head to tail. The total number of islets in a human pancreas has been estimated using morphometrical analysis to vary between 3.6-14.8 million (for review, see Srinivasan *et al.*, 2006). In

general, it is considered that adult pancreatic islets have a complex architecture, with the beta cells being more preferentially located in the islet core and the other cell types being more abundant in the islet periphery (Meier *et al.*, 2006). Islets have a portal circulation, with blood flowing from beta to alpha to delta cells, as well as afferents from the central nervous system, which regulate secretion of these hormones. Alpha and beta cells are exquisitely sensitive to glucose, which stimulates the beta cells and inhibits the alpha cells, thereby maintaining blood glucose levels within a narrow range. The main actions of insulin promote the entry of glucose into tissues and decrease hepatic gluconeogenesis, whereas glucagon stimulates hepatic glycogenolysis when hypoglycaemia threatens (Robertson, 2004).

Type 1 diabetes results from the autoimmune destruction of one islet cell type, the insulin-producing beta cells (Bach, 1994). This involves local production of inflammatory cyto-kines such as IL-1 β , TNF- α , and IFN- γ (Hohmeier *et al.*, 2003). Destruction is by CD4+ and CD8+ T cells and macrophages infiltrating the islets (Foulis *et al.*, 1991).

For over 80 years, the main therapeutic approach to insulin-dependent diabetes has been confined to treating the symptoms by insulin replacement. Pancreatic transplantation has offered a successful therapeutic approach for many years. However, as with all whole-organ transplants, lifelong immunosuppression is required and donor organs are in short supply. Therefore, most pancreas transplantations are conducted in diabetic patients with severe late-stage complications and undergoing kidney transplantation and immunosuppression (Narang and Mahato, 2006). Pancreas transplantation is, therefore, not available to a vast majority of diabetic patients as a therapeutic option.

ISLET TRANSPLANTATION

Methods of islet isolation. The two main islet isolation methods used are "semi-automated" and "manual" (for review, see Paget *et al.*, 2007). Both methods rely on prompt and careful removal and transfer of the donor pancreas to allow isolation to commence, preferably within eight hours.

The technique of islet isolation, with minor variations among the centres, has not changed significantly for several years (Wiseman and Gill, 2004). Islets are isolated by a collagenase-based enzymatic digestion of the donor pancreas with a "Ricordi" chamber (Ricordi *et al.*, 1988), followed by a purification step involving modification of the COBE 2991 cell processor to provide a Ficoll-based continuous density gradient. Besides the Edmonton protocol, the Kyoto islet isolation method is also used (Matsumoto *et al.*, 2007).

The pancreas is procured with the use of the same techniques that are used to procure pancreas for whole-organ transplantation. Pancreas decontamination with antiseptic agents (polyvidonum-iodine, cefazoline, amphotericine B etc.) reduces the risk of infection of the final islet preparation (Bucher et al., 2005; Kin et al., 2007a). Thereafter, the pancreatic duct is cannulated, and collagenase is infused to separate islets from exocrine and ductal tissue (Ricordi et al., 1988; 1990). The pancreas is placed into a chamber (Ricordi chamber), and a continuous digestion process is started to progressively disassemble the organ into fragments of decreasing sizes. Cooling and dilution protect the islets leaving the chamber from further enzymatic action (Ricordi, 2003). Crude Clostridium histolyticum collagenase is widely used for the enzymatic degradation of pancreatic intercellular matrix of collagen (Johnson et al., 1996). Islets are usually isolated using a protocol of Liberase (type of collagenase) perfusion (Linetsky et al., 1997), gentle mechanical dissociation, and purification by densitygradient centrifugation to separate the small islet fraction from the predominant exocrine and non-islet tissue (Lakey et al., 2002).

A major obstacle to successful human islet isolation has been the variability of the enzymatic digestion phase (Kin *et al.*, 2007b). Protease enhances the degradation of all four major components of the extracellular matrix: collagen is degraded more completely, while proteoglycans, glycoproteins and elastin are degraded at a higher rate. Prolonged dissociation in the presence of protease results in a dramatic decrease in islet yield, which correlates with the observation that the enzyme accelerates islet disintegration (Wolters *et al.*, 1992).

Quality of islet preparations. The overall process of islet isolation, purification, preservation, and quality control poses serious challenges to the clinical outcome of islet transplants. Success of human pancreatic islet isolation depends largely on the techniques used during pancreas procurement and the quality of the gland. J.R. Lakey et al. (1996) described donor factors that were associated with successful islet isolation. This report identified a high donor body mass index, older donor age, and absence of cardiac arrest as characteristics associated with higher islet yield. Islet preparations that are clinically satisfactory for transplantation have been achieved in only 50% of pancreases (Morris and Monaco, 2005). Warm ischemia of the pancreas of any duration during organ procurement may be detrimental to subsequent islet yield and functional viability of the islets (Lakey et al., 2002).

The recognised in vitro predictive parameter of graft function is islet preparation quality, which includes not only beta cell mass, but also islet viability and integrity, islet preparation composition and islet insulin secretion. Product release criteria for transplantation include the following: (a) Gram stain-negative for bacteria; (b) purity > 30%; (c) viability > 70%; (d) endotoxin content < 5 EU/ kg of recipient; and (e) packed tissue volume < 10 ml (Frank et al., 2004). However, there are no absolute criteria to define whether an islet is suitable for transplantation in diabetic patients. For example, the level of purification of a successful islet preparation is controversial. Complete purification of islet tissue is probably not necessary and it is likely that islet non-betacells are not essential for successful islet transplantation (Gray, 1989; King et al., 2007). However, the eventual benefits of contaminant cells have not yet been proven. At this stage, the evaluation of insulin responsiveness to glucose in isolated islets also is not justified as a control quality test predictive of graft function (Bertuzzi and Ricordi, 2007). It seems that donor age could represent a determinant of quality, because young age is associated with better islet insulin responsiveness to glucose and graft function in transplanted patients (Ihm et al., 2006).

Islet processing facilities with good manufacturing practice (GMP) provide an ultraclean environment for the safe production of clinical grade islets (Kin *et al.*, 2007a). Most centres operating an active clinical islet transplant programme obtain 300–600 000 islet equivalents (IE)/ pancreas after purification, which is about 50% of the islet tissue content (for review, see Srinivasan *et al.*, 2006). Islet culture before clinical transplantation ensures the transplantation of only the more robust cells and is emerging as standard practice in larger volume centres. It allows the transplant procedure to be performed under elective conditions, with earlier establishment of immunosuppression (Fraud *et al.*, 2005). Islet engraftment. Islets are like an organ in themselves with extensive intraislet vasculature, formed of fenestrated capillary endothelial cell lining, which is essential for the supply of oxygen and nutrients to the cells in their inner core (Menger et al., 1994). This vasculature is disrupted during the process of islet isolation and culture, which causes an accumulation of endothelial fragments and compromises perfusion of the core of islets. Therefore, rapid revascularisation is crucial for islet engraftment, survival, and function post-transplantation (Brissova et al., 2004). Successful islet grafts have been observed to regenerate the microvasculature within 10 to 14 days of transplantation (Menger et al., 1994; Merchant et al., 1997; Beger et al., 1998). Controlling apoptosis during organ procurement, islet isolation/culture, and engraftment also is a potential key to the success in clinical human islet transplantation (Davalli et al., 1996). For example, it has demonstrated that insulin, when administered at the appropriate dose, has potent antiapoptotic effects in human islets. These effects are mediated via effects on pancreatic and duodenal homeobox 1 (Pdx1) and involve changes in the expression of unexpected proteins in the pancreatic islet (Johnson et al., 2006).

Allotransplantation of the islets of Langerhans. Historically, the field of clinical islet transplantation began in earnest from 1974 to 1996. In these attempts, generally less than 10% of type 1 diabetic islet recipients achieved insulin independence (Burke et al., 2004; Gruessner and Sutherland, 2004; Wiseman and Gill, 2004). Injection of donor islets into the liver, although less invasive than whole-organ transplantation, was found to have mixed success, until researchers in Edmonton, Canada presented the so-called "Edmonton protocol" and successful outcome in seven patients with type 1 diabetes mellitus using a glucocorticoidfree immunosuppression regimen (Shapiro et al., 2000). Severe recurrent hypoglycaemia is considered the main indication for islet transplantation today (Morris and Monaco, 2005). With the use of a combination daclizumab, sirolimus and tacrolimus and islets from more than 1 donor pancreas per recipient, success rates of 80% at one year and 20% at five years have been reported (Ryan et al., 2005a). The ideal candidate for islet cell transplantation in its current form is an insulin-sensitive person with type 1 diabetes, who has poor subjective recognition of hypoglycaemia and is experiencing recurrent severe hypoglycaemic episodes, despite optimised medical treatment (Ryan et al., 2004; Srinivasan et al., 2006).

Islet transplantation involves the isolation of functional islets from cadaveric, multiorgan donors. Pancreases from living donors have also been utilised successfully (Matsumoto *et al.*, 2004; 2005; Amiel and Rela, 2005). However, living-donor islet transplantation remains controversial because of the potential for diabetes induction or other morbidities in a healthy donor (Truong *et al.*, 2005). The islets are injected into the hepatic portal vein of the diabetic patient, from where they deposit in well-perfused liver sinuses (Rossini *et al.*, 1999). The procedure should be carried out two or three times (Serup *et al.*, 2001). Numerous clusters of islet cells staining strongly for insulin and glucagon have been shown to be detected within portal triads on biopsy specimens (Farney *et al.*, 1991).

Islet transplantation differs from other tissue and organ transplantation approaches in being a heterotopic graft, located on a site other than the natural location of the tissue. Implantation of islets into the liver or peritoneum rather than the pancreas not only achieves physiological function but may also enhance the chances of graft survival (Rossini et al., 1999). Usually, islets are not transplanted homotopically in the pancreas of the recipient because the pancreas is a highly sensitive tissue. Any injury or manipulation of the pancreas leads to severe pancreatitis, with accompanying pain and tissue destruction (Morrow et al., 1984). However, the pancreas can be considered also as an islet transplant site. The pancreas could offer the advantage of requiring fewer islets than other conventional sites, thereby increasing the possibility that one donor pancreas may serve one or more recipients (Stagner et al., 2007).

Islet xenotransplantation. One promising alternative is xeno-islets for transplantation, and the focus has rested on the feasibility of porcine islets for this purpose. If suitable for transplantation into humans, porcine islets may offer a sustainable islet source. Although there have been reports of success, none have reported consistent success with the use of this approach to transplant islets across species (Robertson, 2004). Xenotransplantation suffers from enhanced immune destruction of transplanted tissue due to xenoantigen rejection. Pig islets, in particular, have been widely studied for a variety of reasons, including: (a) the fact that humans had been treated with pig insulin for more than 60 years; and (b) the fact that pig islets respond to glucose in the same physiological glucose range as human islets (Rother and Harlan, 2004). Recent studies showed no evidence of porcine viral or retroviral infection and demonstrated live encapsulated porcine islets from a type 1 diabetic patient 9.5 years after intraperitoneal transplant of alginateencapsulated porcine islets. These islets were found in abundant nodules throughout the peritoneum (Elliott et al., 2007).

Indications for islet transplantation and clinical outcomes. The challenges to successful transplantation of islets include: (a) isolation, culture, characterisation, and preservation of islets; (b) inflammation and autoimmune-mediated destruction and alloimmune rejection of transplanted islets; (c) failure to revascularise; (d) low transplanted mass and high metabolic demand on the tissue; and (e) a limited supply of islets (Narang and Mahato, 2006). Both the quality and the number of islets affect success rates. The islet mass requirement for transplantation is reflected not only in the achievement and maintenance of normoglycaemia in transplant recipients, but also in terms of long-term graft survival and function (Rickels *et al.*, 2005). The amount of $\geq 10~000$ IE per kilogram of body weight has been considered as an adequate islet mass, which has yielded improved islet function in recipients with type 1 diabetes with hypoglycaemic unawareness (Shapiro et al., 2000).

The function of transplanted islets has often been defined on the basis of C-peptide values, the presence of which in before-transplant C-peptide-negative patients seems to be the best function marker for engrafted islets (Ryan et al., 2005b). C-peptide values as well as the exogenous insulin requirements of recipients were shown to be directly correlated with the number of transplanted islets, but there are many exceptions to this association (Nano et al., 2005; Keymeulen et al., 2006; Bertuzzi and Ricordi, 2007). This data could be partially explained by a difference in quality of islets. C-peptide is also correlated with the reduction of exogenous insulin requirement. Some researchers consider that success of islet transplantation may be best defined by a set of metabolic criteria, not just glucose concentration/metabolism alone (Luzi et al., 2001). The transplant outcome has often been quantified through reaching insulin independence (Hering et al., 2005; Ricordi et al., 2005) or, when this is impossible, through the decrease of insulin requirement as percentage of initial need or reduction of absolute values of insulin units (Ryan et al., 2001; Nano et al., 2005). Records of glycaemic levels could also include the frequency of hypoglycaemic episodes that have been described as greatly decreased after islet transplantation (Ryan et al., 2004). The Edmonton group has proposed beta score as a new parameter, to evaluate the following factors on a scale 0 to 2: fasting glycaemia (<100, 100-126, >126 mg/dl), HbA(1c) (≤6.1, 6.2-6.8, >6.9%), insulin requirement (0, 0.01-0.24, >0.24 units/kg), and stimulated C-peptide (>0.8, 0.3–0.8, <0.3 ng/ml). This provides a simple scoring system that encompasses glycaemic control, diabetes therapy, and endogenous insulin secretion (Ryan et al., 2005b), but this new approach is not yet validated. Recently, a group of Italian researchers has developed a new index called transplant estimated function (TEF) which is easy obtained from daily insulin requirement and HbA(1c) (Caumo et al., 2008).

Islet transplantation can provide metabolic stability for patients with type 1 diabetes. Clinical data produced by some centres on patients 1 year after the transplant report that the percentage of normal C-peptide secretion is 100% and that of insulin independence 80%, with an improvement of glycaemic compensation (Hering et al., 2005; Ricordi et al., 2005). However, the comprehensive percentage of success (in terms of insulin independence) is only ~50% (Shapiro et al., 2006) and the efficacy of a transplant preparation is variable and not always predictable. The benefits of islet transplant in recipients emerge progressively during the early months after transplantation. However, the most extreme example is a patient who became insulin independent 11 months after transplant (Bertuzzi et al., 2002). However, even modest restoration of insulin secretion in islet transplant recipients may result in improved insulin sensitivity and free fatty acid dynamics (Rickels et al., 2006).

In general, islet transplantation is effective at improving glycaemic control and hypoglycaemia unawareness in the short to medium term. However, problems with long-term safety of immunosuppression, islet-induced thrombosis and early detection of loss of islet function remain to be addressed (O'Connell *et al.*, 2006). The challenge is to continue to improve early results and to try to sustain cell function in the long term. Opportunities lie ahead with the development of potentially "islet-friendly" immunosuppressants, the possibility of tolerance induction, new sources of islets, and improvements in islet yield and implantation (Srinivasan *et al.*, 2006).

Islet transplantation in Latvia. From 1983 to 1988, investigations on transplantation of pancreas islet cell culture were carried out also in Latvia in the Department of Transplantation, Medical Academy of Latvia (Rozental *et al.*, 1996). Pancreatic islets were obtained from fetal porcine pancreas, cultivated for 10–12 days and then injected in the lateral part of the abdominal muscle. In total, 187 transplantations were carried out in 144 recipients at the age of 15–60. Elevated levels of insulin and C-peptide were observed. The clinical effectiveness of transplantation continued for a period of 10–12 months (Лейниекс, 1989).

In 2006–2007, in cooperation of Latvian researchers and physicians 17 human pancreata from cadaveric donors were procured and processed for islet isolation again. Successful isolations were carried out by a collagenase-based enzymatic digestion of the donor pancreas both, with a manual method and using a Ricordi chamber, followed by enrichment of the released islets on a density gradient.

ISLET REGENERATION

Taking into consideration the limitation of the quantity of cadaveric donor organs and not always adequate quality of isolated beta cells the development of methods of islet regeneration stimulation is of great importance.

Pancreatic progenitor cells. Islet coculture with pancreatic ductal epithelial cells has been shown to be useful for maintaining islet viability and function after isolation. Long-term culture as well as cryopreservation was shown to decrease the viability of human pancreatic islets, which was prevented by coculture with ductal epithelial cells (Gatto *et al.*, 2003). It was also shown that after clinical islet transplantation, the insulin secretory response to glucose, a measure of the functional beta cell mass, correlates significantly with the number of duct cells originally present in the islets transplanted (Street *et al.*, 2004). Currently, pancreatic ductal epithelial cells have been considered as putative stem cells for islets and an essential component of the extracellular matrix, which plays an important role in secreting appropriate growth factors that support islet viability.

During the past years, progress has been made in the definition of new strategies to produce mature pancreatic beta cells. Different cell sources have been tested for their capacity to differentiate into beta cells. The generation of islets and/or insulin-producing cells of human origin (regeneration therapy) have been explored (Scharfmann, 2003). Xenotransplantation suffers primarily from enhanced immune destruction of transplanted tissue, but the use of replicating cell lines has significant safety concerns for human applications (Narang and Mahato, 2006). Two major potential postnatal sources of new beta cells can be discerned. One is represented by stem cells and the other can involve a range of proposed transdifferentiations from previously mature cells, such as acinar, ductal, mesenchymal, and islet cells (Bouwens and Rooman, 2005).

The presence of beta cells in patients with long-standing type 1 diabetes, despite ongoing autoimmunity, implies that new formation of beta cells may be occurring (Meier et al., 2005). Regeneration of beta cells is therefore an area of active investigation, with recent studies reporting differentiation of pancreatic and nonpancreatic progenitors as well as replication of existing islet beta cells (for review, Gillespie, 2006). Duct tissue from human pancreas can be expanded in culture and then be directed to differentiate into glucose responsive islet tissue in vitro (Bonner-Weir et al., 2000). Several groups have focused on promoting differentiation of islet progenitor and ductal cells into insulin-secreting tissue (Halban, 2004), whereas others believe that other differentiated cells, such as adult small hepatocytes, may be a suitable alternative to isolated human islets for transplantation (Nakajima-Nagata et al., 2004; Chen et al., 2005). M. Zalzman et al. (2003) has developed an immortalised transfected foetal hepatocyte cell line expressing Pdx1 activated multiple beta cell genes, and these cells demonstrate physiological glucose-responsive insulin secretion sufficient to reverse diabetes in mice. It was found that Pdx1-VP16 is able to selectively convert hepatic cells into pancreatic endocrine precursor cells. However, complete transdifferentiation into functional insulin-producing cells requires additional external factors, including high glucose or hyperglycaemia (Cao et al., 2004). It is estimated that activation of Pax4 in Pdx1-VP16-expressing cells reprogrammes pancreatic precursor-like cells into glucose-responsive, more mature insulin-producing cells (Tang et al., 2006). Thus, transdifferentiation of hepatocytes into functional insulin-producing cells may serve as a viable therapeutic option for patients with type 1 diabetes (Cao et al., 2004).

The ability of tissues to generate and maintain the correct number of cells still is a fundamental problem in biology. In principle, tissue turnover can occur by the differentiation of stem cells, as it is well documented for blood, skin and intestine, or by the duplication of existing differentiated cells. However, the extent to which adult stem cells actually participate in these processes in vivo is not clear. Thus, preexisting beta cells, rather than pluripotent stem cells, are the major source of new beta cells during adult life and after pancreatectomy. Terminally differentiated beta cells retain a significant proliferative capacity in vivo and cast doubt on the idea that adult stem cells have a significant role in beta cell replenishment. It has been suggested that cells along the pancreatic ducts may act as progenitors for new islet cells in the postnatal period, although recent lineage tracing experiments suggest that the bulk of newly generated beta cells in adult mice result from the replication of pre-existing beta cells (Dor *et al.*, 2004).

To date, the best candidates for adult pancreatic stem or progenitor cells are 1) duct cells; 2) exocrine tissue; 3) nestin-positive islet-derived progenitor cells; 4) neurogenin-3-positive cells; 5) pancreas-derived multipotent precursors; and 6) mature beta cells (Soria *et al.*, 2005). A population of stem cells from human cord blood also have been successfully induced into insulin-producing islet-like structures, which co-express insulin and C-peptide (Sun *et al.*, 2007b). Regardless of the origin of newly generated beta cells, these cells will need to function in other organs besides the pancreas and will need to be protected from rejection and autoimmune destruction (Soria *et al.*, 2005).

Neogenesis of beta cells. Currently, efforts are being made to differentiate beta cells from precursor populations and to expand beta cells in vitro to generate an unlimited supply of beta cells for transplantation. Theoretically, the same could be done in vivo to expand a patient's existing or transplanted beta cell population. Beta cell mass is increased by beta cell neogenesis, beta cell proliferation, and beta cell hypertrophy (increased cell size), and is decreased by beta cell death, primarily through apoptosis, and beta cell atrophy (decreased cell size). From embryogenesis to adulthood, there is a net increase in beta cell mass as the organism's size increases. A significant portion of the population is predisposed to beta cell failure, for currently unknown reasons. It is likely that factors that regulate beta cell proliferation may play a role, although whether the factors that regulate beta cell mass expansion are the same as those that regulate beta cell mass maintenance is unclear (Ackermann and Gannon, 2007). During adulthood, beta cells proliferate at a low rate, which may gradually decline with age. Approximately, 1–4% of beta cells replicate per day in rats between 30 and 100 days old (Finegood et al., 1995), and 1% of beta cells replicate per day in mice at 1 year of age (Teta et al., 2005). While autoimmune and pharmacological destruction of insulin-producing beta cells is often irreversible, adult beta cell mass does fluctuate in response to physiological cues including pregnancy and insulin resistance (Nir et al., 2007). As far as we know, proliferation of postnatal islet cells is relatively low compared with tissues such as intestines or bone marrow, except in states favouring robust beta cell growth, such as pregnancy, insulin resistance, and obesity (Bouwens and Rooman, 2005; Heit et al., 2006). Experimental data indicate that adult human pancreatic islet cells can be expanded by three serial passages while maintaining their endocrine properties and can yield functional islet-like cell clusters through aggregation in alginate-poly-L-lysine microcapsules that reverse hyperglycaemia in diabetic mice (Tsang et al., 2007). It has been also demonstrated that foetal pancreatic cells differentiate and function normally when placed within barium alginate microcapsules and transplanted (Foster et al., 2007).

Even if beta cell neogenesis does not occur endogenously, it may be experimentally inducible *ex vivo*. Non-endocrine epithelial cells of the adult human pancreas (presumably ductal or acinar) can be induced to adopt a beta cell fate by signals from foetal pancreas (Hao et al., 2006). Transducing cultured human duct cells with Ngn3 activates many islet-specific genes, including insulin, albeit at low levels (Heremans et al., 2002; Gasa et al., 2004). Similar findings in the rat (Dudek et al., 1991) suggest that an embryonic differentiation programme can be reactivated in adult pancreatic ducts, consistent with the overlap in gene expression that is seen between mature ducts and embryonic progenitors (Pierreux et al., 2006). As ducts and acini are currently discarded during human islet isolation, the prospect of spinning this dross into gold is highly attractive (Murtaugh, 2007). Some studies show that pre-existing pancreatic acinar cells contribute to acinar cell, but not islet beta cell, regeneration (Desai et al., 2007) while others demonstrate that insulin-secreting cells can be generated by transdifferentiation from pancreatic acinar cells of rodents in the diabetic state and further suggest that pancreatic acinar cells represent a potential source of autologous transplantable insulin-secreting cells for treatment of type 1 diabetes (Okuno et al., 2007). Also, purified duct cells from adult human pancreas can differentiate to insulin-producing cells (Yatoh et al., 2007).

It is known that many stimuli influence beta cell neogenesis from pancreatic duct cells in vitro and in vivo (Bouwens and Kloppel, 1996). These include growth factors, such as transforming growth factor alpha (TGF-a), epidermal growth factor (EGF), and keratinocyte growth factor. Extracellular matrix also promotes beta cell differentiation from duct cells. In addition, gastrointestinal peptides, such as glucagon-like peptide-1 and gastrin, can stimulate beta cell neogenesis. For example, there are shown that combination therapy with EGF and gastrin increases beta cell mass in adult human pancreatic islets in vitro and in vivo, and this appears to result from the induction of beta cell neogenesis from pancreatic exocrine duct cells (Suarez-Pinzon et al., 2005). It was found that enzymatic dissociation of pancreatic tissue itself leads to activation of EGF-signalling, and that inhibition of EGF receptor kinase blocks the transdifferentiation (Minami et al., 2005). Even a modest attenuation of EGF receptor signalling leads to a severe defect in postnatal growth of the beta cells, which leads to the development of diabetes (Miettinen et al., 2006). Stem cells with a mesenchymal phenotype have been recently shown to develop in human islet cultures in vitro by dedifferentiation of epithelial beta cells induced by powerful growth factors like EGF or fibroblast growth factor (FGF). This phenomenon is named epithelial to mesenchymal transition. The islet-derived mesenchymal cells express nestin and vimentin and are able to redifferentiate into insulin-producing cells given the appropriate stimuli (Gershengorn et al., 2004).

Markers of pancreatic differentiation. Pancreatic islets contain a distinct population of cells that express the stem cell-specific marker nestin, an intermediate filament protein (Lendahl *et al.*, 1990). These cells derived from islets have properties of stem cells and can differentiate in culture into cells with liver, pancreatic exocrine/ductal, and endocrine

phenotypes (Zulewski et al., 2001). The isolated nestin- and Isl1-positive cells display a mesenchymal phenotype as mirrored by their ability to differentiate into adipocytic and osteocytic phenotypes given the appropriate stimuli. These cells are negative for insulin and the insulin promoter factor 1 (Ipf1). Upon differentiation with serum free medium supplemented with a cocktail of differentiation stimulating factors they could be induced to form islet-like cluster and to express several pancreatic developmental genes. This includes the transcription factors Ipf1, Isl1, Pax4, Pax6, Ngn3, Nkx2.2 and Nkx6.1, as well as the pancreatic endocrine genes insulin, glucagon and somatostatin. Immunocytochemistry and electron microscopy also identified glucagon and C-peptide positive cells. Unfortunately, the actual differentiation efficacy is limited and the cells are not yet able to secrete insulin in response to glucose (Eberhardt et al., 2006).

Stem cells with the potential to differentiate into insulinproducing cells have been also described in the liver (Sapir *et al.*, 2005), the central nervous system (Hori *et al.*, 2005), the spleen (Kodama *et al.*, 2003) and bone marrow (Ianus *et al.*, 2003; Oh *et al.*, 2004). Human bone marrow-derived mesenchymal stem cells (MSC) were described to express at a low level the islet transcription factor Nkx6.1 and to differentiate into insulin-expressing cells upon adenoviral transduction with vectors encoding the transcription factors Ipf1, Hlxb9 or Foxa2 (Moriscot *et al.*, 2005). MSC were also shown to bear the potential to adopt a neural phenotype *in vitro* and *in vivo* suggesting a neuro-endocrine developmental capacity of these cells (Woodbury *et al.*, 2000).

At present, it is shown that MSC from diabetes mellitus patients can differentiate into functional insulin-producing cells under certain conditions in vitro. Using a diabetic patient's own MSC as a source of autologous insulin-producing cells for beta cell replacement would be feasible (Sun et al., 2007a). Moreover, recent investigations have shown that portal vein transplantation of bone marrow MSC transdifferentiated into islet-like cells could alleviate the hyperglycaemia of diabetic rats (Wu et al., 2007). However, treatment with sirolimus and tacrolimus, immunosuppressants used in the Edmonton protocol for human islet transplantation, inhibited beta cell regeneration and prevented the normalisation of glucose homeostasis. This suggests that regenerative therapy for type 1 diabetes may be achieved if autoimmunity is halted using regeneration-compatible drugs (Nir et al., 2007).

Regeneration therapy strategies. Regeneration therapy is classified into three categories: 1) *in vitro* regeneration therapy using transplanted cultured cells, including embryonic stem cells (ESC), pancreatic stem cells, and beta cell lines, in conjunction with immunosuppressive therapy or immunoisolation; 2) in *ex vivo* regeneration therapy, patients' own cells, such as bone marrow stem cells, are transiently removed and induced to differentiate into beta cells *in vitro*; 3) in *in vivo* regeneration therapy, impaired tissues regenerate from patients' own cells *in vivo*. It is very likely that a pharmacological treatment resulting in regeneration of beta

cells would be inefficient in type 1 patients due to the expected recurrence of autoimmunity and inflammatory destruction of the newly formed beta cells. Thus, the challenge regarding beta cell mass control is to find application in cell therapy (*ex vivo* generation) or in regenerative therapy, controlling the immune system represents the major challenge (Bouwens and Rooman, 2005). Beta cell neogenesis from non-beta cells and beta cell proliferation *in vivo* has been considered, particularly as regeneration therapies for type 2 diabetes.

Regeneration therapy of pancreatic beta cells can be combined with various other therapeutic strategies, including islet transplantation, cell-based therapy, gene therapy, and drug therapy to promote beta cell proliferation and neogenesis, and it is hoped that these strategies will, in the future, provide a cure for diabetes (Yamaoka, 2002).

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AIZKUŅĢA DZIEDZERA SALIŅU TRANSPLANTĀCIJA UN REĢENERĀCIJA CUKURA DIABĒTA ĀRSTĒŠANAI

Saslimstība ar cukura diabētu dažādu iemeslu dēļ (iedzimtība, neveselīgs uzturs, mazkustīgs dzīves veids u.c.) pēdējos gados palielinās gan Latvijā, gan visā pasaulē. Gandrīz vienmēr šiem pacientiem ir jāuzsāk insulīna terapija. Tajā pašā laikā jaunākās modernās tehnoloģijas ir sekmējušas jaunu, alternatīvu metožu ieviešanu arī cukura diabēta ārstēšanā. Viena no tām – Langerhansa saliņu transplantēšana, kura ir salīdzinoši vienkārša un relatīvi maz invazīva, salīdzinājumā ar visa aizkuņģa dziedzera transplantēšanu. Šī metode varētu būt īpaši piemērota 1. tipa diabēta pacientu ārstēšanā, kad insulīnu ražojošās beta šūnas ir gājušas bojā un glikozes līmenis asinīs ir palielināts (hiperglikēmija). Klīniska saliņu transplantēšana, īpaši pēdējos gados, imūnsupresijai neizmantojot kortikosteroīdus, ir bijusi sekmīga vairākos saliņu transplantēšanas centros gan Eiropā, gan arī citur pasaulē. Saliņu izolēšanas pamatā ir donora aizkuņģa dziedzera enzimātiska sadalīšana ar kolagenāzi "Rikordi" kamerā, kam seko attīrīšanas procedūra Fikola gradienta centrifugācijā. Rakstā apkopota informācija par saliņu transplantēšanas un izolēšanas problēmām un sasniegumiem, kā arī klīniskajiem rezultātiem. Apskatīta arī beta šūnu reģenerācija, ieskaitot to neoģenēzi. Sniegts īss pārskats par Latvijas zinātnieku pieredzi saliņu transplantēšanā.