Cell Therapy in Type 1 Diabetes

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Abstract

The incidence of diabetes mellitus has grown exponentially in the last few years. Etiopathogenesis of diabetes implies a β-cells damage in the islet of Langerhans, either through an autoimmune reaction present in type 1 diabetic patients or through altered function within these cells that affect their ability to secrete a properly functioning insulin hormone, in patients suffering from type 2 diabetes.

Exogenous insulin supply is, at the moment, the therapy of choice of the disease but it does not allow tight control of glucose regulation, leading to long-term complications. Over the past few decades, pancreas or pancreas-kidney organ transplantation has been the most effective treatment for severe diabetic patients. Recently, an alternative promising therapeutic approach, consisting of successful pancreatic islet transplantation to reconstitute the insulin producing β cells, has also emerged. Unfortunately the number of donor islets is too low compared to high number of patients needing a transplant, so the search for new renewable sources of high-quality β-cells becomes highly topical.

In this review, starting from the description of state of art of islet transplantation, we summarize the more recent promising approaches to the generation of new β-cells giving a big encany to adult stem/progenitor cells.

Keywords: Type 1 diabetes cell therapy; Pancreatic progenitor cells; Islet transplantation; Transdifferentiation of exocrine pancreatic cells.

Abbreviations: ADSCs (adipose derived stem cells): BM (bone marrow): EPCs (endothelial progenitor cells): ESRD (end stage renal disease): ESCs (embryonic stem cells): IAK (islet transplantation after kidney grafting): HGF (hepatocyte growth factor): hPDMSCs (human placenta derived mesenchymal stem cells): HSCs (hematopoietic stem cells): IPCCs (insulin producing cell clusters): IPSs (induced pluripotent stem cells): MSCs (mesenchymal stem cells): STZ (streptozotocin).

Introduction

The goal of therapy of type 1 diabetes (DM-1) is to restore a glyco-metabolic picture as close as possible to normal.

Since the cause of type 1 diabetes is the failure to produce insulin due to the destruction of β-pancreatic cells, the therapy is represented by the replacement of lost endocrine function. Since 1921, when Nicolae Constantin Paulescu, first in the world, was able to cure diabetes, having discovered insulin which he called Pancreina, for most patients the replacement therapy is the exogenous insulin supply. It has long been searching for therapeutic solutions able to ‘cure’ diabetes permanently, that is to replace the β-pancreatic cells, freeing patients from daily insulin requirements.

The more radical solution is the whole pancreas transplant, a surgical procedure developed in the last ten years, which has reached success rates comparable to those of other organ transplants (80% of transplanted patients achieved insulin independence and maintained for more than 6-8 years) [1].

However, pancreas transplantation is a challenging surgical procedure, requires a significant immunosuppressive therapy to prevent organ rejection and, therefore, takes place only in combination with a kidney transplant in diabetic patients with ESRD (end stage renal disease) on dialysis [2].

In the last few years islet transplantation has been developed as an alternative promising therapy but, more recently, experimental approach consisting of stem cells administration, transdifferentiation of ductal cells or genetic reprogramming seem the future of diabetes cell therapy.

Pancreatic Islet Transplant

General considerations

The islets of Langerhans are clusters of endocrine cells and constitute about 1% of pancreatic tissue where they play a function of sensing glucose blood levels, secreting hormones that regulate them.

The pancreatic islets for transplantation are prepared by an isolation procedure, developed by Camillo Ricordi, which consists of a combined mechanical and enzymatic digestion of the pancreas, followed by a purification step through gradients of different density (Ricordi Chamber) (Table 1).

The possibility to culture pancreatic islets offers a range of therapeutic opportunities aimed at improving the efficiency and graft survival, which also is made with a technique much simpler than that required for vascularised pancreas transplantation. This procedure is associated with low morbidity and can be repeated, to give an additional dose of islets, when it is necessary to achieve adequate metabolic control and insulin independence.

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The first islet transplants performed in patients with type 1 diabetes mellitus (DM-1) and under immunosuppressive therapy for organ transplantation, have been characterized by a low success rate, expressed in terms both of insulin independence and recovery of c-peptide secretion, due to the inconsistent quality of the transplanted islets and inadequate immunosuppression [3]. The revival of this procedure occurred with the development of an innovative scheme of immunosuppression, known as the 'Edmonton Protocol', and its application in selected centers in the world able to reproducibly obtain high quality islets [4,5]. The first results were excellent, with a success rate at one year of almost 100%, although, with continued experience, these decreased dramatically, especially at 2-3 years, but these data were of extraordinary importance because they helped to stimulate research in the field of β-cell replacement.

**Benefits of islet transplantation**

The goal of islet transplantation is to control blood sugar levels, restoring β-cell function. The tight control of blood glucose, obtained by administering intensive insulin therapy, has shown great benefits in preventing or delaying the progression of micro and macro-vascular chronic complications of diabetes, but these benefits are associated with an increased risk of acute severe hypoglycaemia [6,7].

The islet transplantation could be considered a valuable therapeutic option to achieve a short-term metabolic control in patients with DM-1 with frequent and severe episodes of hypoglycemia without prodromal symptoms.

Immediately after the infusion of islets, also in cases of sub-optimal cell mass, it happens a dramatic reduction in daily insulin requirement, which is associated to an improvement in glycemic control, demonstrated by normalization of glycated hemoglobin, improvement in the c-peptide levels and insulin secretion during metabolic tests [8].

Although clinical trials, made in the 80s and 90s, have shown that graft function (defined clinically as the persistence of measurable levels of c-peptide in the blood) can be sustained over time, the insulin-graft function (defined clinically as the persistence of measurable levels in the c-peptide levels and insulin secretion during metabolic tests [8].

Tab 1: Procedure for isolation of pancreatic islets

<table>
<thead>
<tr>
<th>Procedure for isolation of pancreatic islets</th>
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<tbody>
<tr>
<td>Pancreatic islet cells are obtained from multorgan donors after separation from the tissue surrounding the gland</td>
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<tr>
<td>The Wirsung duct is cannulated to allow injection of a solution containing collagenase in order to relax the organ and enable the achievement of an effective enzyme activity during the dissociation</td>
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<td>The gland is then divided into several parts and transferred to the dissociation chamber, consisting of a lower portion, where the pancreas is inserted together with metal balls, and an upper portion consisting of an inverted funnel, separated by a porous metal filter with a specific porosity</td>
</tr>
<tr>
<td>The digestion process is the combination of the enzymatic effect (obtained by increasing the temperature) and mechanical (generated by the action of the ball during the stirring of the room) that results in the release of fragments of the pancreas that can pass through the filter metal due to the unidirectional flow of liquid dissociation</td>
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<tr>
<td>The digested pancreas is, then, purified by using different density gradients (continuous and discontinuous) in order to enrich the endocrine component</td>
</tr>
<tr>
<td>The islets that make up only 1% of pancreatic tissue can be viewed using a dye that gives it a distinctive red color</td>
</tr>
<tr>
<td>After the purification enriched fractions containing levels of purity can be obtained</td>
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Recent clinical studies have shown that insulin independence can be achieved after repeated infusions of islets, using immunosuppressive protocols without steroids [9,10].

The normalization of glycated hemoglobin and insulin-independence are generally obtained after transplantation of a sufficient number of islets (>13,000 islet equivalents / kg recipient body weight) in a single infusion or repeated infusions [11,12].

Other studies have reported insulin independence in 80% of patients at one year, when a protocol of sequential islet transplantation, in order to reach a sufficient mass, was performed [13].

Data suggest that transplantation provides a better metabolic and physiological control compared to insulin treatment; in particular islet transplantation allows a long-term prevention of severe hypoglycemia also when an insulin supportive therapy is required to maintain stable blood sugar levels (in the case of insufficient mass of transplanted islets or partial engraftment of them) [14,15].

The effects of islet transplantation on the progression of diabetes complications are currently under investigation, but it has been shown an improvement of left ventricular ability and no increase in carotid wall thickness during a three years follow-up post-transplant. An improvement in renal function after IAK (islet transplantation after kidney grafting), was also observed in patients with chronic renal failure and DM-1. Another interesting observation is the increase of blood flow in central retinal artery and vein [16].

**Limits of islets transplantation**

There are still many obstacles to overcome before achieving a successful islet transplantation in humans. There are several factors to consider to optimize pancreatic islets transplantation:

- Cold storage of pancreas before islet isolation should be less than eight hours;
- The quantity of islet cells: it should be transplanted a minimum of 6000 IE/kg per weight of the recipient (where IE indicates islet equivalents: an expression of the volume of islets, converted to the number of islets with a diameter of 150 micrometers);
- Administration of antilymphocyte antibodies at the time of islet transplantation;
- Site of the transplant: currently liver is preferred;
- Adverse effects of immunosuppressive therapy based on the use of corticosteroids;
-Recurrence of the autoimmune processes associated with the DM-1;
- Ischemic processes that may affect the islet cells themselves.

**Transplant of encapsulated islets**

A variation to islet transplant, which is under experimentation, consists of their encapsulation with a selective, natural and non-immunogenic membrane that allows free transport of oxygen, nutrients and hormones, but particularly a tolerance of humoral and cellular immune system against these "masked" islets. This would eliminate or reduce the need of immunosuppressive therapy [17,18].

This membrane is a polysaccharide, sodium alginate, which, in contact with islet cells in a solution of calcium chloride, manages...
to wrap. The following islets transplantation is performed in the peritoneum [19,20].

**Islets/stem cells combined transplant**

Experimental data indicate that bone marrow transplant leads to a state of chimerism (coexistence of immune cells of the donor with those of the recipient) which can re-educate the recipient’s immune system to accept transplanted organs or tissues from the same donor without the need of immunosuppressive therapy. Stem cells could reconstitute the immune system of the donor into the recipient, “teaching” the recipient to accept the islet from the same donor [21]. These experiments have already proved effective in experimental animal models, but not in clinical trials.

**Stem Cells: A Source for new β-Cells**

**Pancreatic stem cells**

The postnatal pancreatic duct may harbour islet precursor/stem cells because of specific cellular migrations during embryonic development [22]. Islet neogenesis, the generation of new islets from pancreatic stem/progenitor cells located in ducts, could be an active process in the postnatal pancreas; interestingly insulin-producing cells can be generated from adult pancreatic ductal tissues in vitro [23-28]. Fluorescent activated cell sorting, which allows characterization of human β-cells through CD95 [29], could be also a promising technique for isolation of multipotent pancreatic progenitors from both neonatal and adult pancreata. By combining flow cytometry and clonal analysis [30,31], some authors described molecular markers expressed specifically by possible pancreatic stem/progenitor cells candidate as the hepatocyte growth factor (HGF) c-Met. Moreover, they identified a newly specific marker for ductal cells, CD133 [31].

The use of adult stem cells isolated from patients could solve immunological problems associated to cell transplant. Unfortunately, adult stem cells are rare and difficult to expand in culture. In contrast, it has been reported that new generation of β-cells, after birth, could take origin from existing cells, and not from putative pancreatic stem cells [30]. Insulin-producing β-cells are also produced from endogenous endocrine progenitors following injury [31]. The existence of adult pancreatic stem/progenitor cells and their ability to proliferate in response to particular stimuli should be more investigated.

**Stem cells of no pancreatic origin**

Recent studies have demonstrated that embryonic stem cells (ESCs) [32-34], induced pluripotent stem cells (iPSCs) [35,36], and adult stem cells form bone marrow (BM) [37] pancreas [38,39], liver [40], umbilical cord blood [41], Wharton’s jelly [42], placenta [43], could differentiate into insulin producing cells. Because of their high pluripotency ESCs could be ideal for islet regeneration, but obviously their use is under debate for ethical/legal issues and risks of teratoma formation [44].

The seminal work regarding the potentiality of ESCs to differentiate to insulin secreting structure similar to pancreatic islets was published by Lumelsky and co-workers [32]. In this work authors identified a highly enriched population of nestin-positive cells from embryoid bodies (EBs) as possible candidates for pancreatic islets generation. EBs were plated into a serum-free medium to achieve a negative selection for all cell types different from nestin-positive cells. These cells were then expanded in the presence of a mitogen, basic fibroblast growth factor (bFGF), in N2 serum-free medium, followed by mitogen withdrawal to promote cessation of cell division and differentiation.

Using a RT-PCR (reverse transcription polymerase chain reaction) authors showed that the ES cells processed following their protocol expressed GATA-4, a marker of definitive and visceral endoderm, HNF3b, a marker of definitive endoderm, as well as markers of pancreatic cell fate, including the murine insulin 1, insulin II, islet amyloid polypeptide (IAPP), and the glucose transporter-2 (GLUT2). Glucagon, a marker for the pancreatic α cells, was also induced in differentiated cells. The acquisition of a pancreatic fate was also confirmed by immunocytochemical analysis which showed insulin staining after mitogen withdrawal, resulting in many strongly insulin positive cells by the end of the processing.

The ability of ES cell–derived islet-like cell clusters to survive and function in vivo was tested through grafting cell clusters subcutaneously in the shoulder of streptozotocin-diabetic mice. Implanted cells vascularized, remained immunoreactive to insulin and formed aggregates morphologically similar to normal pancreatic islets. Authors did not observe a sustained correction of hyperglycemia, although grafted animals were able to maintain their body weight and survived for longer periods of time than hyperglycemic sham-grafted controls.

Boyd and colleagues [45] published a work which raised doubts about the Lumelsky’s protocol. The first was about the interpretation of gene expression data regarding the pancreatic markers; Boyd et al. infact assert that it should be defined whether the gene expression was attributable to a single cell within the isolated cluster or whether all cells in the cluster had transcribed the genes. Furthermore, Lumelsky’s data showed that “insulin producing cell clusters” (IPCCs) had a mixed pancreatic phenotype because they express not only β-cells markers but also those of α-cells.

Another weakness in Lumelsky’s protocol emerged from quantitative PCR data [45], which demonstrated that expression of insulin-1 and insulin-2 mRNA was consistently higher in IPCCs obtained by Blyszczuk and coworkers [46] compared to Lumelsky-generated clusters, implying that the Blyszczuk protocol was capable of generating IPCCs with superior de novo insulin-producing activity. The amylase-2 expression in IPCCs obtained by other groups following Lumelsky’s protocol [47] suggested that the protocols may promote also exocrine differentiation; this evidence was not in favor of a unique β-cell phenotype acquired by ESCs. Rajaogopal et al, observed also, though immunofluorescence, an attenuated relative ratio of c-peptide to insulin suggesting that the insulin content of IPCCs is an unequal combination of de novo synthesis and adsorption from the culture medium [48]. Most notably, however, the Blyszczuk protocol [46] consistently produced the highest level of c-peptide indicating that this protocol was capable of superior de novo synthesis of insulin. Furthermore cell clusters obtained from Lumelsky and Blyszczuk showed prominent glucagon containing [45] with insulin indicating, that IPCCs derived from ESCs may not surrogate β-cells but may, in contrast, more closely resemble a hybrid of α and β cells. Boyd et al. proposed that glucagon/insulin costaining could alternatively indicate that IPCCs derived from ESCs were developmentally immature endocrine cells as suggested by an high expression of neurogenin-3 which, instead, is downregulated in fully mature islets [49]. Authors suggested also that IPCCs could be defective in their glucose-sensing capacity in vivo. This contention was supported by the glucose stimulation assays that showed that
IPCCs released insulin in response to minimal glucose stimulation (3.3 mmol/l glucose) but did not release significant amounts of insulin at higher glucose stimulation (25 mmol/l glucose).

In light of the aforementioned issues it seems that ESCs based strategy to obtain pancreatic islet like cells needs further investigations.

A valid alternative to ESCs could be adipose derived stem cells (ADSCs) because of their abundance, availability and possibility to be used for autologous transplant [50]. In this regard at least three different research groups showed that ADSCs could, under specific culture conditions, differentiate into insulin, somatostatin, and glucagon expressing cells or c-peptide positive cells [51,52]. In particular Chandra and coworkers [53] packed these cells in immunoisolation capsules and tested their in vivo/in vitro functionality demonstrating their ability to restore normoglycemic conditions in streptozotocin-induced diabetic mouse. Interestingly this study showed, for the first time, that also undifferentiated ADSCs were able to determine a moderate control of blood glucose levels, leading to the speculation that the autocrine and paracrine factors of regenerating pancreas and hyperglycemic local diabetic micro-environment of mice may contribute to ADSCs differentiation. It is worthy of noting that such a phenomenon it has also been shown for bone marrow derived stem cells [54].

Islet neogenesis from the constitutively nestin expressing human umbilical cord matrix derived mesenchymal stem cells has also been reported. Kadam and co-workers [55] showed that human placenta derived mesenchymal stem cells (hPDMSCs) could differentiate in islet-like cells able to restore normoglycemia when transplanted under the kidney capsules of streptozotocin-induced diabetic mice. Interestingly a gene expression profile of undifferentiated hPDMSCs showed, unlike human cord blood or amnion derived mesenchymal stem cells, that they express mRNA for insulin, glucagon, somatostatin, Ngn3, and Isl1.

**Stem cells as a support for islet function and regeneration**

Vascularisation of pancreatic islets is important for their ability to secrete insulin. Obviously islet isolation [56] needs a break of vascularisation which could compromise irreversibly endocrine function; some preservation methods counteract this deleterious effect [57,58].

Endothelial cells deliver oxygen and nutrients to endocrine cells, and contribute to create a microenvironment for beta-cells function. In particular, they can induce insulin gene expression during islet development, stimulate beta-cell proliferation, and produce a number of vasoactive, angiogenic substances and growth factors [59-61].

Published works reported the ability of hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), and endothelial progenitor cells (EPCs) to contribute to islet revascularization through vessel formation by differentiation into mature endothelial cells (EPCs) or release of proangiogenic factors such as hepatocyte growth factor (HGF) and vascular endothelial growth factors A [62-64]. In this regard Mathew et al [65] provided evidence that bone marrow derived EPCs, transplanted after a pancreatic injury, migrate to the site of damage helping the recovery of injured beta-cells even if they do not differentiate in insulin-producing cells.

Two other papers stress the concept of a synergism between β-cells and bone marrow (BM) derived cells. Sakata et al [66] showed that only a combined transplant (under the kidney capsule) using total bone marrow derived cells (not only mesenchymal cellular fraction) and islets was able to significantly lower blood glucose levels in a streptozotocin-induced diabetes murine model; interestingly in the case of transplant of BM cells alone there were no normoglycemic mice and no insulin-positive cells, suggesting that a direct differentiation of BM cells in beta-cells was unlikely. Ito and colleagues [67] provided similar evidence but in this case the site of combined BM cells/islets transplantation was liver and not kidney.

BM derived stem cells could also have a role in stimulation of β-cells regeneration [68-70]. For example, it has been shown that infusion of mesenchymal stem cells in NOD/SCID mice, after STZ-induced islet destruction, could increase the number of endogenous β-cells improving hyperglycemia.

**Beyond Stem Cells based Therapy: Transdifferentiation of Adult Cells towards an Islet like Phenotype**

The pancreas development starts with dorsal and ventral protrusions of the primitive gut epithelium that fusing later to form the definitive organ characterized by the appearance of glucagon-producing cells (Figure 1). Then a few insulin-producing cells appear, often co-expressing the glucagon hormone. A later step consists of a peak of endocrine cell genesis leading to the generation of numerous fully differentiated insulin-expressing β-cells and glucagon-producing α-cells. At the end of pancreas development endocrine cells begin to form well-organized islets of Langerhans.

The mechanisms responsible for the development of these different endocrine cell types are not fully understood, but experiments consisting of generation of mice deficient for a number of pancreatic transcription factors helped to shed more light about them identifying Sox9, Pdx1, Ngn3, IA1, Pax4, Arx, Nkx2.2, Nkx6.1, Nkx6.2, Pax6, and MafA as crucial mediators of organ development [71].

All pancreatic cells derive from Pdx1-expressing progenitor cells [72] and this recent information was at the basis of a lot of experiments...
which allowed obtaining pancreatic cells from other cell types [73-75]. In all these cases it was achieved an adeno-viral-mediated misexpression of Pdx1 in mouse liver observing a prevention of streptozotocin-induced hyperglycemia in animal models. Besides this, Kaneto et al [76] showed that concomitant adeno-viral application of two factors, Pdx1 and Ngn3 or NeuroD in the liver of mice caused a transdifferentiation of hepatic cells into insulin-producing cells associated to a significant amelioration of glucose-tolerance.

Intriguingly, also exocrine pancreatic cells have the capacity to generate their endocrine counterpart when exposed in vitro to a particular microenvironment consisting of agonists of the JAK2/STAT3 signalling pathway as epidermal growth factor and leukemia inhibitory factor [77]. We report also our experience in a porcine model about the isolation, by a surgical microdissection, of Wirsung duct cells which, upon simple in vitro exposure to glucose, acquired the ability to secrete insulin and glucagon [28]. Finally it seems worthy of note the finding that human monocytes treated with macrophage colony-stimulating factor and interleukin 3 can transdifferentiate in pancreatic islet-like cells in a glucose dependent manner [78].

Conclusions

Regenerative medicine is one of the fields of research which has undergone the greatest development in the last few years. Undoubtedly diabetes cell therapy, for the enormous social and economical implications, is one of the most investigated branches of regenerative medicine.

The first therapeutic approaches, consisting of pancreas transplant and, more recently, islet transplantation, showed significant limits so alternative approaches based on a full knowledge of cellular and developmental biology of pancreatic cells have been under full consideration in the last years. Transdifferentiation, the process which lead an adult cell to change its phenotype into another, seems a promising approach given that adult cells are autologous and are not dangerous or potentially tumorigenic and free of ethical implications as, instead, embryonic stem cells.

In conclusion, despite promising experimental published data and regardless of the strategies to increase availability of β-cells, the transfer of the experimental results to the clinic still seems far away.

Conflict of Interest Statement

All authors declare the absence of any employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants which could inappropriately influence their work.

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