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Perspectives of Cell Therapy in Type 1 Diabetes

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1. Introduction

Type 1 diabetes is an autoimmune disease leading to the destruction of pancreatic β cells. The reduction of β cell mass results in insulin deficiency that leads to a failure of glucose homeostasis with increased levels of glucose in blood. Hyperglycemia which *per se* is detrimental for the organism and may be life-threatening, may in the long term associate with chronic complications involving blood vessels and nerves. The gold standard treatment for diabetes patients aimed to reach a tight control of glycemia, relies on intensive insulin therapy based on multiple daily injections or continuous subcutaneous infusion of insulin. A tight control of glycemia reached with such regimens was shown to significantly reduce the incidence of microvascular complications in respect to the conventional insulin therapy. Nevertheless, to reach an optimal control of blood levels may prove to be difficult when compared to the physiological condition where this is guaranteed by the pancreatic β cells (Suckale, 2008). Therefore, preservation of β cell mass could be an important therapeutic target to reduce microvascular complications and to improve the glycemia control (Gonez & Knight, 2010). It is generally accepted that the endocrine pancreas has some regenerative capabilities, although it is still debated which cells are involved in β cell turnover. In rodents the capability of adult pancreas to increase β cell mass has been documented in physiological conditions and after injury. The understanding of mechanisms involved in β cell turnover may therefore be relevant to design new therapeutic strategies aimed to maintain a β cell mass or to favour regeneration of β cells. These strategies however, should take to account the problem of recurrent autoimmunity that in type 1 diabetes not only impairs the original β cell mass, but may also limit the regenerative process. Indeed, autoimmune T lymphocytes may kill the β cells newly formed in response to injury (Fan & Rudert, 2009).

2. β cell regeneration: Contribution of stem/progenitor cells or replication of β cell?

The physiological turnover of the long-lasting endocrine cells of pancreas requires the generation of new cells even with a very slow kinetic.

The organ growth after birth requires a coordinated increase in the number of constitutive cells. Moreover, in the adult body most of the tissues and organs have the ability to replace the cells that die for physiological senescence or following limited injury. The source of newly formed cells may derive from resident stem/progenitor cells or from the ability of differentiated cells to re-entry into cell cycle and replicate themselves. The prevalence of these two mechanisms varies in different organs and tissues; however, they may result in the restoration of the original tissue conformation and function. We therefore should expect that also the endocrine pancreas retains the ability of regeneration in appropriate physiological conditions. The nature and the location of the cells involved in such processes, as well as the mechanisms involved in the activation of the regenerative processes, still remain largely unknown.

The concept of "stem cell" implies the ability of unlimited self renewal and of high multilineage differentiation potential into different types of mature cells. Therefore, stem cells play fundamental roles in organogenesis during embryonic development, and in the adult are responsible for the growth, homeostasis and repair of many tissues.

In the haematopoietic system, the intestine and the skin, tissues that require a high cell turnover, the stem cells are critical for maintaining their homeostasis. However, adult stem/progenitor cells are present in the majority of tissues and organs of mammalian organisms, including the central nervous system (Reynolds & Weiss, 1992), retina (Tropepe et al., 2000), skeletal muscle (Jackson et al., 1999), liver (Herrera et al., 2006) and kidney (Bussolati et al., 2005).

In tissues with a low rate of cell turnover, such as the kidney, the lung, the skeletal muscle and the liver, the resident stem cells may activate after injury and participate in tissue repair. Tissue resident stem cells preferentially generate differentiated cells of the tissue of origin, suggesting a relevant role in the postnatal growth of organs, in physiological turnover and in tissue repair. Tissue-resident adult stem cells are thought to co-localize with supporting cells within specific regions or specialized microenvironments in each tissue/organ, called stem cell niche (Jones & Wagers, 2008; L. Li et al., 2005; Moore & Lemischka, 2006). In bone marrow the haematopoietic stem cells (HSC) are located in the endosteal niche, associated with the osteoblasts of the inner surface of the cavities of trabecular bone that could provide factors able to regulate number and function of HSC (Mitsiadis et al., 2007) and in the perivascular area of sinusoids that could ensure homeostatic blood cell production and prompt responses to haematological stresses (Kiel et al., 2005). The other stem cells present in bone marrow are the mesenchymal stem cells (MSC), undifferentiated adult stem cells of mesodermal origin, which localize in perivascular areas in the bone marrow in close association with HSC (Shi & Gronthos, 2003) and that have the capacity to differentiate into cells of connective tissue lineages, including bone, fat, cartilage and muscle (Y. Jiang et al., 2002).

Other stem cell niches detected in mammals (da Silva Meirelles et al., 2008; L. Li & Xie, 2005) are the epithelial stem cell niche in skin that resides in the bulge area of the hair follicle beneath the sebaceous gland (Cotsarelis et al., 1990; Niemann & Watt, 2002; Sun et al., 1991), the intestinal stem cell niche located at the fourth or fifth position above the Paneth cells from the crypt bottom (Booth & Potten, 2000; He et al., 2004; Sancho et al., 2004) and the neural stem cell niche at the subventricular zone and the subgranular zone of the hippocampus where neural stem cells could reside and support neurogenesis in the adult brain (Doetsch et al., 1999; Temple, 2001).

A problem in the identification of tissue resident stem cells is that we do not know specific markers allowing tracing of pluripotent stem cells in various tissues. Therefore, resident stem cells are mainly defined by functional *in vitro* assays using cultured cells, and their *in vivo* exact localization and function remains elusive. Several studies suggest that the adult tissue resident stem cells belong to the MSC lineage (da Silva Meirelles et al., 2006). The minimal criteria to define human MSC established by *Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy* (da Silva Meirelles et al., 2008; Dominici et al., 2006), include cell positivity for CD105, CD73, and CD90 and negativity for CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR as well as osteo-, chondro-, and adipogenic-differentiation capabilities.

A perivascular location for MSC has been suggested, correlating these cells with pericytes providing an explanation for the presence of MSC virtually in all vascularized tissues (da Silva Meirelles et al., 2006). The perivascular zone may act as a MSC niche *in vivo*, where microenvironment factors may modulate their phenotype with transition to progenitor and mature cells. For many years the concept of niche has been associated with a hierarchical nature of stem cells that undergoing asymmetric division insure self-renewal and generation of a progeny with progressive loss of proliferative potential and gain of differentiated characteristics (Till et al., 1964). More recently, a continuum model of stem cell biology has been proposed (Colvin et al., 2004; Quesenberry et al., 2005). It has been postulated that the phenotype of stem cells is labile, it varies with position in the cell cycle and that it is reversible (Colvin et al., 2007). This cell-cycle reversibility is at the basis of the continuum model of stem cell biology, in which the phenotype of stem cells is reversibly changing during the cell cycle transit awaiting for a terminal-differentiating stimulus at a cycle-susceptible time. In this model the status of the cell cycle and the exposure to environmental factors play critical roles in the acquirement of different phenotypes by the same cell in different functional states (Quesenberry et al., 2007). Recently, Quesenberry and Aliotta proposed that the so-called niche consists in areas of influence which are continually adjusting to individual circumstances (Quesenberry & Aliotta, 2008). Based on these considerations, the refined regenerative system in mammalians does not need to position in each organ different stem cell types but it would be sufficient to maintain few undifferentiated cells with self-renewal capability that depending on the circumstances may vary their phenotype to replace the loss of differentiated cells. On the other hand, differentiated cells may re-acquire an undifferentiated phenotype and re-entry in to cell cycle first to restore the cell mass and subsequently to re-differentiate and restore functional integrity. In this context, the exchange of genetic information among cells by microvesicles in a defined environment plays a critical role in modulating plasticity of stem cells as well as the response of differentiated cells to injury (Deregibus et al., 2010).

Studies on β cell proliferation in humans are limited, but there is evidence that this process occurs at relatively high levels in the first 2 years of life declining thereafter with the possibility, at least in animals, of re-induction under conditions of insulin-resistance, such as pregnancy or obesity. (Meier et al., 2008). This suggests that β cells may retain an intrinsic capacity to replicate.

In the adult, endocrine pancreas β cells are considered to have a very low turnover. However, albeit quite slowly, β cells undergo senescence and should be continuously replaced by newly formed cells. By combining abdominal CT scans and morphometric analysis of human pancreatic tissue, Meier et al reported that the β cell mass expands by

several fold from birth to adulthood as result of an expansion in size of islets rather than an increase in number of islets (Meier et al., 2008). The increase in β cell number per islet mainly occurs in young children in coincidence with the growth of the organ size. Cnop et al provided evidence for a long lifespan and low turnover of human islet β cells estimated by mathematical modelling of lipofuscin accumulation (Cnop et al., 2010). Human β cells, unlike those of young rodents, are long-lived and in the adult human β cell population is mainly established in the first 20 years of life. Dor et al using a method for genetic lineage tracing to determine the contribution of stem cells to pancreatic β cell neogenesis showed that pre-existing β cells, rather than pluripotent stem cells, are the major source of new β cells during adult life and after pancreatectomy in mice (Dor et al., 2004). These results suggest that terminally differentiated β cells retain, at least in mice, a significant proliferative capacity *in vivo*. Nir et al used a transgenic mouse model to study the dynamics of β cell regeneration from a transiently induced diabetic state (Nir et al., 2007). Lineage tracing analysis in this model indicated that enhanced proliferation of surviving β cells played the major role in regeneration. These studies provided evidence that adult pancreatic β cells are formed by self duplication rather than stem cell differentiation (Dor et al., 2004; Nir et al., 2007). On the other hand there are studies suggesting that regenerated β cells derive from precursors located within pancreatic ducts in the proximity of islets (Juhl et al., 2010). The origin from these precursors has been demonstrated in rodent models of pancreatic damage (Bonner-Weir et al., 2004; Xu et al., 2008). Monitoring the expression of Neurogenin 3 (Ngn3), the earliest islet cell-specific transcription factor in embryonic development, Xu et al showed activation of β cell progenitors located in the ductal lining in injured adult mouse pancreas (Xu et al., 2008). They found that differentiation of the adult progenitors is Ngn3 dependent and generates all islet cell types, including glucose responsive β cells that proliferate, both *in situ* and when cultured in embryonic pancreas explants. This study suggests that multipotent progenitor cells present in the pancreas of adult mice can increase the functional β cell mass by differentiation and proliferation rather than by self-duplication of pre-existing β cells only. Li et al investigated whether after partial pancreatectomy in adult rats, pancreatic-duct cells serve as a source of regeneration by undergoing a dedifferentiation and redifferentiation (W.C. Li et al., 2010). The Authors detected after pancreatectomy an early loss by the mature ducts of the ductal differentiation marker Hnf6, followed by the transient formation of areas composed of proliferating ductules, called foci of regeneration. These ductules expressed markers of the embryonic pancreatic epithelium Pdx1, Tcf2 and Sox9 (W.C. Li et al., 2010). Since foci subsequently form new pancreatic lobes, it was suggested that these cells act as progenitors of the regenerating pancreas. Islets in foci initially resemble embryonic islets as they transiently expressed the endocrine-lineage-specific transcription factor Ngn3 and lacked of MafA expression and contained low percentage of β cells. The numbers of MafA(+) insulin(+) cells progressively increased with the maturation of foci (W.C. Li et al., 2010). Based on these observations, it was suggested that adult pancreatic duct cells may recapitulate aspects of embryonic pancreas in response to injury (W.C. Li et al., 2010). This mechanism of regeneration implicates the plasticity of the differentiated cells within the pancreas.

As schematized in figure 1, after injury β cells may be replaced by replication of β cells or from differentiation of stem cells (SC) localized within the islets or in exocrine pancreatic tissues (ductal and acinal cells). After extreme loss of β cell mass, glucagon producing α cells may transdifferentiate in β cells. A possible contribution to β cell neogenesis comes from

bone marrow derived stem cells of both haemopoietic (BM-derived HSC) and mesenchymal (BM-derived MSC) origin. These cells act by a paracrine mechanism releasing factors that favour tissue repair. Moreover, transdifferentiation of ductal and acinal cells may generate insulin secreting cells.

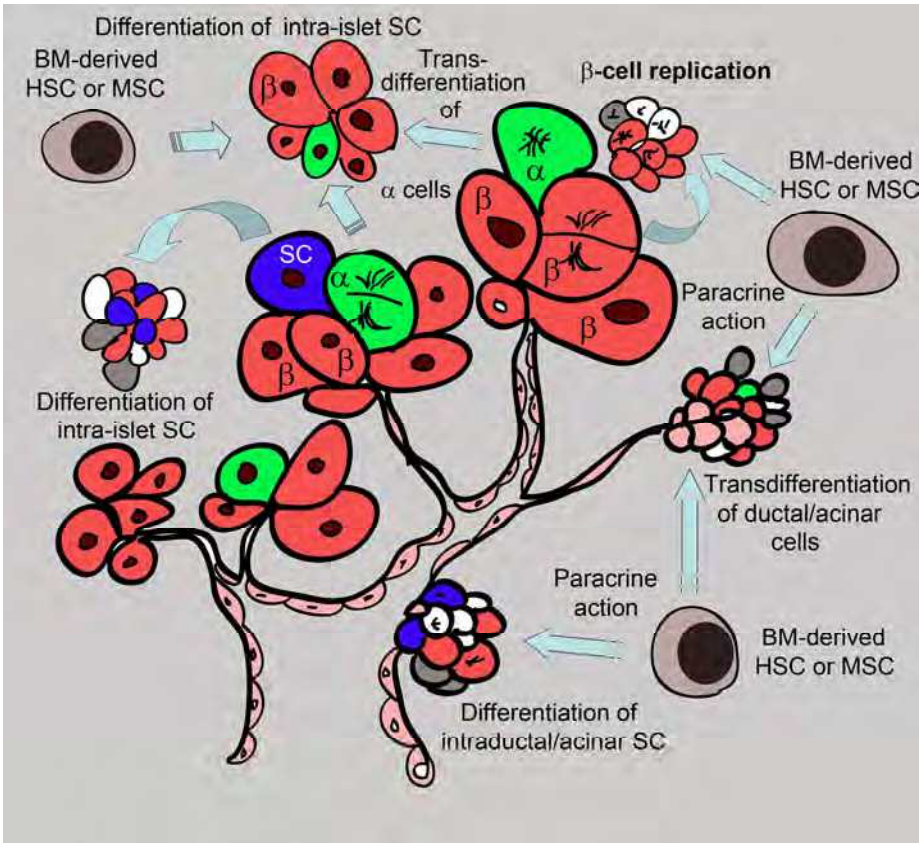

Fig. 1. Mechanisms potentially involved in β cell neogenesis.

The discrepancy in identification of cells that in the adult sustain β cell neogenesis, may result from the differential contribution in condition of physiological turnover and in condition of injury where the regenerative processes are accelerated and cells other than insulin producing β cells are involved to insure a reparative process. The study of Thorel et al showing the conversion of adult pancreatic α cells into β after extreme β cell loss stands in this line of interpretation (Thorel et al., 2010). In this study the Authors used *in vivo* genetic approaches to obtain near total β cell loss without autoimmunity combined with cell lineage tracing. For this purpose, they created a model of inducible, rapid β cell removal (>99%) by administration of diphtheria toxin (DT) in transgenic mice in which the DT receptor was expressed only in β cells (Thorel et al., 2010). In transgenic mice the systemic administration of DT permitted a specific cell ablation by apoptosis (Saito et al., 2001). In this model, β cell regeneration was monitored in combination with cell lineage tracing to investigate the

origin of newly formed β cells. The results obtained showed that the adult pancreas can generate new β cells after their near total loss, mainly by the spontaneous reprogramming of glucagon-secreting α cells (Saito et al., 2001). Therefore, β cell replication may account for maintaining homeostasis, whereas transdifferentiation of other cell types could be required after injury to replace the lost cells.

Summarizing these studies, one can envisage a scenario in which β cells may replicate themselves to maintain homeostasis (Dor et al., 2004), tissue specific precursors localized within pancreatic ductal cells generate α and β cells after pancreatic injury and glucagon secreting α cells can transdifferentiate into β cells to repair an extremely severe selective β cell loss. Translation of these experimental studies in humans may be difficult without the help of lineage tracing or of specific cellular markers.

3. β cell mass in type 1 and type 2 diabetes

Despite the evidence of some turnover in adult humans and the apparent capacity of endocrine pancreatic β cells to regenerate throughout life, patients with type 1 as well as type 2 diabetes have substantial deficit in β cell mass, approximately 99% in long-standing type 1 diabetes and 65% in long-standing type 2 diabetes. This loss of β cells implies that restoration of endogenous insulin secretion might be accomplished through replacement or regeneration of islet cells.

The β cell deficit in type 1 diabetes is related to the established autoimmune destruction of the target cells. However, several lines of research indicate that some β cell regeneration may occur in recent onset type 1 diabetic patients (Willcox et al., 2010) and even in patients with long-standing type 1 diabetes (Meier et al., 2005). The failure of β cell regeneration in type 1 diabetes may be related to the increased vulnerability of the newly forming β cells to apoptosis induced by inflammatory cytokines (Meier et al., 2006).

Other mechanisms, involved in all forms of diabetes, include the hyperglycemic induction of the nitric oxide synthase (NOS)-dependent mechanisms in islet microendothelium, with production of the vasoactive mediator nitric oxide (NO) (Suschek et al., 1994), endothelial cell loss and vasculature disruption (Zanone et al., 2008). NO is in fact increased in hyperglycemic conditions, and has an established direct islet cytotoxicity and potentially impairs insulin release (Steiner et al, 1997; Suschek et al., 1994; Kröncke et al., 1993). Islet microendothelial cells are also source of the proinflammatory cytokine IL-1β under hypeglycemic conditions, independently of any viral or immune-mediated process. IL-1β impairs insulin release in human islet, induces Fas expression enabling Fas-mediated apoptosis and IL-1β is thus implicated as a mediator of glucotoxicity (Maedler et al., 2001; Loweth et al., 1998). The metabolic mechanisms by which hyperglycemia initiates apoptosis in vascular endothelium are incompletely understood. These mechanisms include oxidative stress, increased intracellular Ca++, mitocondrial dysfunction, changes in intracellular fatty-acid metabolism, and impaired phosphorylation of the protein kinase Akt (Favaro et al., 2008). Akt signaling pathway plays a pivotal role in preventing apoptosis in a variety of settings (Datta et al., 1999), and, in particular, Akt activation is crucial for the ability of factors such as insulin, IGF-I and VEGF to inhibit apoptosis in cultured endothelium (Jung et al., 2000). Recent data highlight the Akt role also in insulin-mediated glucose transport and pancreatic β cell mass and function (Bernal-Mizrachi et al., 2004; Elghazi et al., 2006). As for type 2 diabetes, the progressive increase in glucose levels that characterizes its natural history has been claimed to be due to gradual reduction of function and mass of β cells, and

a significant reduction in β cell mass is clearly established in Type 2 diabetes (Donath & Halban, 2004; Weir & Bonner-Weir, 2004). The same mechanisms of glucotoxicity reported are involved, together with dyslipidaemia. In murine models of type 2 diabetes, short-term hyperglycemia has been shown to increase islet capillary blood pressure and perfusion, in a glucose dependent and reversible pathway, possibly mediated by a NOS–dependent mechanism (Bonner-Weir, 2004; Carlsson et al., 1998). However, with age, persistent hyperglycemia induces islet hypoperfusion. Inducible NOS (iNOS) increases the islet blood perfusion also in prediabetic low dose streptozotocin-treated mice, a model of type 1 diabetes (Carlsson et al., 2000). Increased islet capillary flow and pressure could, over time, contribute to the damage of the islet endothelium and thickening of the capillary wall, thus decreasing the islet perfusion. The hyperglycemia-induced NO production by the endothelium could also result cytotoxic to the islets and directly impair insulin release.
In Zucker diabetic fatty rats, a model of type 2 diabetes, it has been shown that changes in the islet vasculature play a key pathogenetic role in the development of diabetes (X. Li et al., 2006). In a biphasic pattern, an early vascular hyperplasia was followed by endothelial cell loss and vasculature disruption, in parallel to progressive islet failure.

4. Islet transplantation as strategy for β cell replacement

Allogenic pancreatic islet transplantation has become a suitable therapeutic option for the treatment of patients with unstable type I diabetes after the introduction of the Edmonton Protocol based on the optimization of islet isolation techniques and the development of a rapamycin-based glucocorticoid-free immunosuppressive regimen (Ricordi, 2003; Shapiro et al., 2000, 2003, 2006). However, after 5 years only 10% of the recipients were insulin independent (Ryan et al., 2005; Shapiro, 2006). In addition, although a sufficient islet mass can be obtained from good quality pancreata, to achieve insulin independence usually are needed islet preparations derived from multiple donors (Biancone & Ricordi, 2002). Therefore, this procedure is limited by the supply of cadaveric donors. Moreover, several factors may be responsible of the progressive dysfunction of transplanted islets. After an initial islet mass loss following the intraportal infusion, due to an inflammatory reaction, engraftment requires efficient islet revascularization by a chimeric vascular tree formed by host and recipient endothelial cells (Brissova et al., 2004). A poor vascular engraftment is one of the main causes of islet loss. Other factors that concur to islet loss include the exposure of islets to increased lipid levels, a side effect of immunosuppressive therapy based on mTOR inhibitors (Hafiz et al., 2005; Pileggi et al., 2006). Exposure to high-dose of calcineurin inhibitors (CNI) is recognized to induce direct β cell toxicity and functional impairment. The antiproliferative effects of mTOR inhibitors and CNI inhibit tissue remodelling and reduce β cell self-renewal (Nir et al., 2007). Moreover, the anti-angiogenic activity of rapamycin is a potential limitation of the current immunosuppressive protocols, that may be particularly detrimental in the early engraftment phase (Cantaluppi et al., 2006). Therefore, to overcome these problems it is necessary to improve the recovery and quality of islet cells from a single-donor pancreas and to develop strategies allowing the inhibition of inflammatory reactions, the improvement of islet vascularization and of islet engraftment using safer and less cytotoxic immunomodulatory approaches (Mineo et al., 2009; Pileggi et al., 2006; Ricordi, 2003). Recently, increased islet yields have been obtained by improving techniques of pancreas recovery and preservation and of islet isolation and purification (Pileggi et al., 2006;Ricordi, 2003). On the other hand, peritransplant interventions based on

combination therapies have been proposed to inhibit allo- and auto-immune response minimizing the side effects and favoring development of T regulatory cells (Treg) to maintain long term tolerance and to favor β cell regeneration. Targeting the costimulatory molecules involved in T-cell activation and/or adhesion molecules by immunomodulatory agents now available for clinical applications could be an option to reduce the side effects of immunosuppression and the islet toxicity and to achieve specific immune tolerance (Ricordi & Strom, 2004). Several experimental studies suggest that a combined islet transplant and cell therapy with bone marrow–derived cells, mesenchymal cells, Treg, and tolerogenic dendritic cells may modulate recipient immune response and increase the engraftment and long term survival of islets (Mineo et al., 2008). This possibility is supported by recent clinical trials demonstrating stable mixed haematopoietic chimerism and/or improved tolerance in kidney allograft recipients using nonmyeloablative conditioning and donor haematopoietic stem cell infusion (Sykes, 2009).

Another factor limiting successful islet engraftment is the inflammatory reaction that takes place in the liver after portal vein infusion of islets. This observation led to experiments of co-transplantation of islets with bone marrow-derived mesenchymal stem cells to take advantage of the anti-inflammatory action of these cells (Ito et al., 2010).

To improve islet vascularization is also a must for a better engraftment of islets. It has been shown that bone marrow-derived endothelial progenitor cells (EPC) isolated from peripheral blood specifically localize within sites of endothelial injury inducing a regenerative program. EPC are able to chimerize with donor vessels in transplanted organs, suggesting a putative role of these cells in graft revascularization (Schuh et al., 2008¸ Koopmans et al., 2006). EPC are recruited to the pancreas in response to islet injury and EPC-mediated pancreas neovascularization may facilitate the recovery of injured β cells improving islet allograft function (Mathews et al., 2004). In a murine model of islet transplantation, the increase of EPC in the peripheral circulation obtained by mobilization with granulocyte-macrophage colony-stimulating factor has been associated with higher vascular density and engraftment (Contreras et al., 2003). Therefore, the identification of factors able to enhance neoangiogenesis may increase the success of islet transplantation.

Another goal of combination therapies is to preserve islet function after detection of graft dysfunction (Froud et al., 2008). For this purpose it has been suggested, for instance, the use of exenatide, glucagon-like peptide synthetic analog, anti-tumor necrosis factor α agents or immunomodulatory therapy (Faradji et al., 2008; Froud et al., 2008). However, the recently released results of two trials addressing strategies of combination therapies are disappointing. The phase three trial based on combination of anti-CD25 mAb (Daclizumab) that blocks IL-2 signalling pathway in activated T cells without interferring with Treg, in combination with mycophenolate mofetil that bloks the *de novo* purine synthesis in T and B lymphocytes, reported no improvement of β cell preservation (Gottlieb et al., 2010). The anti-CD25 mAb was tested in another trial in association with exenatide. Also in this trial the improvement of β cell function was not observed (Rother et al., 2009). Several other phase II-III National Institutes of Health (NIH)–sponsored randomized trials in islet transplantation alone and in islet-after-kidney transplantation are currently under evaluation by the Clinical Islet Transplantation Consortium (http://www.citisletstudy.org/). An alternative to allow long term survival of islets after transplantation is the development of efficient encapsulation techniques aimed to guarantee immune-isolation, an adequate exchange of nutrients to islet cells and release of insulin (Calafiore et al., 2006). This kind of approach may protect from cell-mediated rejection of

implanted islets, although soluble mediators may still reach β cells and induce cell death. On the other hand, this strategy might allow full maturation of embryonic stem cells into glucose sensitive insulin secreting β cells. Indeed, human embryonic stem cells differentiated into pancreatic endoderm could be encapsulated in a protective device before transplantation (D'Amour et al., 2005; Kroon et al., 2008). Experiments in NOD mice demonstrated that encapsulated human β cell precursors may generate functional insulin producing cells after implantation improving diabetes (S.H. Lee et al., 2009). As an alternative, ongoing studies are evaluating the use of encapsulated porcine islets to correct type 1 diabetes (Elliott et al., 2007). Preliminary studies in humans showed long term survival of xenotransplantation of porcine neonatal islets and Sertoli cells using a technology to provide an immune protective environment (Valdes-Gonzales et al., 2005). A vascularized chamber model allowing tissue growth in threedimension under influence of hypoxia that triggers angiogenesis has been developped in mouse (Cronin et al., 2004) and rat (Mian et al., 2000; Tanaka et al., 2000). Vascularized chambers containing syngenic islets were used to improve glucose control in diabetic mice (Hussey et al., 2009). In this study islets were transplanted into prevascularized chambers implanted on the epigastric pedicle in the groin of diabetic mice resulting in a significant reduction in blood glucose levels and improvement of glycemic control. This study suggests that islet survival and function are enhanced by prevascularization of tissue engineering chambers before islet transplantation (Hussey et al., 2009). Opara et al. reported a new design of a bioartificial pancreas comprising islets co-encapsulated with angiogenic protein in permselective multilayer alginate-poly-L-ornithine-alginate microcapsules and transplanted in an omentum pouch in diabetic experimental animals (Opara et al., 2010). A great effort is invested to improve the encapsulation techniques to avoid clogging of the device that may reduce the influx of nutrients and glucose and impair the insulin efflux. Teramura & Iwata recently reviewed the obstacles and the new techniques to overcome these problems, such as conformal coating and islet enclosure with cells (Teramura & Iwata, 2010).

In consideration of the shortage of pancreata for islet transplantation and of the side effects of current immunosuppressive protocols, the research has been focused on the possible development of alternative sources of functional β cells. The ideal approach to overcome the current inadequate supply of human pancreatic islet cells for transplantation is the availability of an unlimited source of transplantable insulin-producing cells. The potential of adult and embryonic stem cells to generate islet cells as well as development of appropriate conditions for expansion and differentiation to β cells of pancreatic islet cell precursors or of cells that share common embryonic origin such as liver cells is under intense investigation (Mineo et al., 2009). Finally, xenogeneic islet transplantation remains a viable therapeutic option for the future (Ricordi, 2003).

5. In search for alternative sources of β cells

The consideration that stem cells play a crucial role to self-renewal in physiologic and pathologic conditions in the endocrine pancreas (Bouvens, 2006; Sarvetnick et al., 2007a, 2007b) prompted researchers to develop SC-based therapy to stimulate β cell regeneration.

In the last years, a variety of approaches have been employed to induce β cell neogenesis using pluripotent stem cells (embryonic stem cells or induced pluripotent stem cells), multipotent adult stem cells such as the hepatic oval cells or terminally differentiated cells such as the exocrine pancreatic cells (Yechoor & Chan, 2010). Brolén et al investigated the potential of

human embryonic stem cells to differentiate into β cells (Brolen et al., 2005). They found that signals from the embryonic mouse pancreas induce differentiation of human embryonic stem cells into insulin-producing β cell-like cells. Human embryonic stem cells (hESC) under two-dimensional growth conditions spontaneously differentiated in Pdx1(+)/Foxa2(+) pancreatic progenitors and Pdx1(+)/Isl1(+) endocrine progenitors but not in insulin-producing cells. The differentiation of β cell-like cell clusters required co-transplantation with the dorsal pancreas from mouse embryos. hESC-derived insulin(+) cell clusters exhibited several features of normal β cells, such as synthesis (proinsulin) and processing (C-peptide) of insulin and nuclear localization of key β cell transcription factors, including Foxa2, Pdx1, and Isl1 (Brolén et al., 2005). Insulin-producing islet-like cells were also generated from human embryonic stem cells under feeder-free conditions (Jiang et al., 2007). Cell aggregates formed in the presence of epidermal growth factor, basic fibroblast growth factor, and noggin were finally matured in the presence of insulin-like growth factor II and nicotinamide. The temporal kinetic of pancreas-specific gene expression was considerably similar to that of *in vivo* pancreas development. The final population contained cells representative of the ductal, exocrine, and endocrine pancreas. Ricordi et al reported a diabetes reversal in mice by embryonic-derived stem cells (Ricordi & Edlund, 2008). They showed that endodermal-derived embryonic stem cells were able to differentiate into cells that expressed typical pancreatic endodermal markers and, once transplanted in diabetic mice, these cells showed a diffuse staining for insulin, other hormones and markers of fully differentiated β cells (Ricordi & Edlund, 2008). The therapy with embryonic stem cells represents an exciting approach towards β cell regeneration and function. So far, the most promising data are from a group of NovoCell who demonstrated the generation of glucose-responsive insulin-secreting cells *in vivo* by pancreatic endoderm derived from human embryonic stem cells into immune-deficient mice (Kroon et al., 2008). However, several problems remain to be solved, such as the reproducibility of the advance, the protection of the engrafted cells against tumor formation and immune-protection that could be achieved by encapsulation devices. Recently, Matveyenko et al tried to reproduce and extend the studies of NovoCell by the implantation of human embryonic stem cell-derived pancreatic endoderm into athimic nude rats, analysing the metabolic parameters of insulin sensitivity and glucose-stimulated insulin secretion to verify the development of viable glucose-responsive insulin secreting cells. The implantation was assessed into the epididymal fat pads of the athymic nude rats or subcutaneously into Theracyte encapsulation devices for 20 weeks (Matveyenko et al., 2010). The data resulting from this study not completely confirmed the development of islet-like structures from human embryonic stem cells differentiated to pancreatic endoderm, since the extent of endocrine cell formation and secretory function was insufficient to be clinically relevant given that human C-peptide and insulin were detectable at very low levels, no increase in human C-peptide/insulin levels after glucose challenge and no development of viable pancreatic tissue or efficient insulin secretion by implantation in the encapsulation devices were present (Matveyenko et al., 2010).

Nevertheless, because the use of embryonic stem cells is burdened with the limited access to embryonic tissues and with the complex ethical concerns involved with the use of embryonic cells, more attention has been focused on adult stem cells. We found that stem cells derived from normal adult human liver (HLSC) with multiple differentiating capabilities distinct from those of oval stem cells may generate islet-like structures. HLSC expressed several MSC markers such as CD73, CD90, CD29, CD44 and the stem cell marker nestin (Herrera et al., 2006). At variance of MSC, HLSC did not express α-SMA and expressed liver tissue-specific proteins such as AFP, a marker of hepatocyte precursors, and

human albumin, suggesting a partial hepatocyte commitment. At variance with oval cells, the HLSC isolated from normal adult liver, did not express the CD34, c-kit and CK19 markers, and were pluripotent. Moreover, the HLSC were able to differentiate in appropriate conditions into pancreatic insulin producing cells (Herrera et al., 2006). When cultured in DMEM with high glucose content (23mM) for one month followed by 5-7 days of culture in the presence of 10 mM nicotinamide, HLSC formed small spheroid cell clusters positive for human insulin and Glut2 which is a glucose transporter that has been suggested to function as a glucose sensor in pancreatic β cells (L. Yang et al., 2002). Moreover, HLSC under this differentiating condition, were positively stained with the Zn-chelating agent dithizone, that is specific for the insulin containing granules (Shiroi et al., 2002). It has been recently shown that adult human pancreas contains rare multipotent stem cells that express insulin (Smukler et al., 2011).

Several studies demonstrate that the β cell phenotype can be induced both *in vitro* and *in vivo* by transfection of pancreatic transcription factors into the liver that develops from the same embryological origin of pancreas (Kojima et al., 2003; Lemaigre & Zaret, 2004; Nagaya et al., 2009).

The potential of bone marrow derived MSC has also been explored. Genetically modified MSC by recombinant PDX-1 adenovirus or by non-virus gene transfection, were able to express insulin sufficient to reduce blood glucose in the streptozotocin mouse model of diabetes (Y. Li et al., 2007). The differentiated PDX-1+ human MSC expressed multiple islet-cell genes such as neurogenin3 (Ngn3), insulin, GK, Glut2, and glucagon, produced and released insulin/C-peptide. After transplatation into STZ-induced diabetic mice the differentiated PDX-1+ human MSC induced within 2 weeks euglycemia and maintained it for at least 42 days. These findings suggest that appropriately modified MSC may allow enrichment of human β cells and represent a possible source for cell replacement therapy in diabetes (Y. Li et al., 2007).

Human umbilical cord blood contains several types of stem/precursor cells. Recent studies indicate the possibility to obtain multipotent stem cells from umbilical cord blood able to confer protection to β cells and to stimulate β cell neogenesis (Zhao & Mazzone, 2010). Islet-like cell clusters were obtained from MSC derived from humbilical cord vein. However, they secreted very low amounts of insulin in vitro (Chao, 2008). Denner et al showed that directed engineering of human cord blood stem cells may produce C-peptide and insulin (Denner et al., 2007).

A better understanding of the mechanisms of β cell regeneration may provide further insight for development of cell based therapeutic approaches. Besides the potential contribution of circulating and resident stem cells, the transdifferentiation of existing pancreatic cells such as pancreatic ductal/acinar cells into insulin secreting cells could be potentially exploited (Baeyens et al., 2005; Cantaluppi et al., 2008; Minami et al., 2005; Rosenberg, 1995).

The successful differentiation of stem cells into β cells may prove not to be easy. The secretion of insulin in physiological concentration in response to glucose concentration is the goal to be achieved. One of the more difficult tasks is to maintain an intact coupling of stimulus-secretion in order to obtain a regulated insulin secretion to keep physiological glucose concentration.

Another pre-requisite for β cell replacement therapy is to obtain a non limiting source of cells possibly derived from the patient himself to avoid the allo-immune response (Wagner et al., 2010). The possibility of reprogramming patient's own cells could be an

approach to these requirements. This was rendered possible by the discovery that induced pluripotent stem cells (iPS) can be generated from adult human somatic cells such as fibroblasts by transfection with selected genes encoding for transcription factors able to confer the characteristics of pluripotency to cells (Takahashi & Yamanaka, 2006; Yamanaka et al., 2007). iPS share the same differentiation potential of embryonic stem cells and they can differentiate into cells of different lineages including insulin producing cells. Tateishi et al tried to generate human iPS cells by retroviral expression of human Oct4, Sox2, Klf4 and c-Myc in the human foreskin fibroblast cells and tested the differentiation potential of human iPS cells into insulin secreting islet-like clusters, demonstrating that iPS cells derived from human skin cells can be differentiated into pancreatic islet-like cluster cells. These cluster cells were shown to contain C-peptide-positive and glucagon-positive cells and, more importantly, to release the C-peptide upon glucose stimulation (Tateishi et al., 2008). iPS cells were also generated from patients with type 1 diabetes by reprogramming their adult fibroblasts with three transcription factors (*OCT4, SOX2, KLF4*). The iPS generated from patients were shown to differentiate into insulin-producing cells (Maehr et al., 2009). A proof of principle for potential clinical applications of reprogrammed somatic cells in the treatment of diabetes type 1 or 2 was provided by Alipio et al who were able to reverse hyperglycemia in diabetic mouse models using iPS-derived pancreatic β like cells (Alipio et al., 2010). iPS could overcome the ethical issues and the immunogenicity of embryonic stem cells. The possibility to use iPS individually tailored for each patient is certainly appealing. However, several problems of bio-safety must be solved before iPS enter in clinical use. These include an aberrant or incomplete differentiation and a tumorigenic potential. Reprogramming cells typically requires integration of genes such as *c-MYC, OCT4 AND KLF4* that are known to be oncogenic and may favour development of tumors (Miura et al., 2009). Although after reprogramming the transgenes undergo silencing, it is always possible a reactivation. Therefore, the research in this field is actively searching for alternative strategies to induce pluripotency.

6. Stem cell therapy to counteract the autoimmune destruction of β cells

Assuming that we could generate pluripotent stem cell lines for each individual patient and that after infusion they would be able to restore the β cell mass, we still have to face the problem of recurrent autoimmunity. The autoimmune destruction of islet β cells by reactive T lymphocytes not only plays a role in the establishment of type 1 diabetes but also impairs an efficient regenerative process. Newly forming β cells show increased vulnerability to apoptosis induced by inflammatory cytokines (Meier et al., 2006), explaining the failure of β cell regeneration in type 1 diabetes. Therefore, newly generated β cells are systematically killed as they acquire the mature phenotype, thus impairing the regenerative attempts in type 1 diabetic patients. Furthermore, therapeutic intervention studies in new onset type 1 diabetes involving broadly immunosuppressive agents, such as cyclosporin A, failed to produce lasting remission, demonstrating the inherent tendency of the autoimmune effector response in humans to recur. Therefore, it is critical that any immunemodulatory therapy induces tolerance to β cell antigens, while minimizing detrimental effects on host defence. This is strengthened by recurrence of type 1 diabetes in syngenic pancreas transplantation (Sutherland et al., 1984).

Cell-based therapeutic strategies for immunemodulation that answer to the requirement of safety and effectiveness are under development. The intravenous use of humanized antibodies against CD3 (part of the T-cell receptor complex) soon after the initial onset of diabetic clinical symptoms decreased the insulin requirement compared to controls without this monoclonal antibody therapy. However, this therapy is burdened by several adverse effects (Keymeulen et al., 2005; Herold et al., 2002; McDevitt & Unanue, 2008).

Other approaches of immunemodulation included subcutaneous administration of heat-shock protein (Raz et al., 2001) or intravenous injection of rabbit polyclonal anti T-cell globulin (Saudek et al., 2004). In many of these studies a short term effective preservation of β cell function was observed; however, only few patients no longer required insulin treatment. Using human recombinant glutamic acid decarboxylase (GAD65) as therapeutic vaccine it was observed a preservation of C-peptide levels and a decrease in insulin dose requirement only in patients with very recent-onset of diabetes (Ludvigsson et al., 2008; Uibo and Lernmark 2008). Compelling evidence indicates the relevant role of Treg in the inhibition of autoimmune response. A decreased number and function of Treg have been reported in patients with type 1 diabetes (Roncarolo & Battaglia, 2007). Therefore, intervention to restore Treg function in diabetic patients is an attractive therapeutic strategy. Nevertheless, only few studies have shown re-establishment of Treg function in diabetes. Zhao et al demonstrated the possibility to correct functional defects of CD4+ CD62L Treg using human cord blood stem cells. This strategy allowed reversal of autoimmune diabetes in NOD mice (Y. Zhao et al., 2009) providing proof of principle that cord blood stem cells can correct function of Treg.

Treg may act by producing IL-10 and in particular transforming growth factor-β1 (TGFβ1), cytokines that are known to contribute to the induction of immunetolerance. It has been suggested that protection of newly generated β cells achieved by treatment with Treg depends on the formation of TGFβ1 cell ring surrounding pancreatic islands and conferring protection against autoimmune T lymphocytes inducing their apoptosis (Y. Zhao et al., 2009). In a pilot study in Type 1 diabetic children based on administration of autologous umbilical cord blood shortly after disease onset a lower level of HbA1c and a reduced insulin requirement was reported. However, none of the patients achieved insulin-treatment independency (Haller et al., 2007).

Recent studies raised great interest on the immunemodulatory potential of mesenchymal stem cells (Le Blanc & Pittenger, 2005). MSC can modulate several T cell functions exerting an immunesuppressive effect. MSC that lack MHC class II molecules and do not express key costimulatory molecules B7-1, B7-2, CD40 and CD40L, were shown to reduce the expression of several lymphocyte activation markers (Aggarwal & Pittenger, 2005). The mechanism has been related to the induction of an anti-inflammatory phenotype in dendritic cells, naive and activated T cells and NK cells, and to an increase of the regulatory T cell population. This results not only in the inhibition of T cell proliferation to polyclonal stimuli, but also in inhibition of naive and memory antigen-specific T cells response to their cognate peptide (Krampera et al., 2003). The MSC-induced suppression has been ascribed to several soluble factors, including hepatocyte growth factor (HGF), TGF-β1 and prostaglandin E_2 (PGE_2) (Aggarwal & Pittenger, 2005). Moreover, MSC have been shown to induce mature dendritic cells type 2 (DC2) to increase IL-10 secretion thus promoting anti-infammatory DC2 signaling. Several studies suggest that MSC improve the outcome of allogenic transplantation and hamper graft-versus-host disease (Le Blanc et al., 2004; Ringden et al.,

2006). Studies on the immunemodulatory potential of MSC in human type 1 diabetes are still lacking but it has been reported their regenerative potential in diabetic NOD/SCID mice leading to an increased number of pancreatic islets and β cells (R.H. Lee et al., 2006). We recently demonstrated that human allogenic bone marrow derived MSC can abrogate *in vitro* a pro-inflammatory Th1 response to islet antigen GAD in new onset type 1 diabetes, by impairing the production of IFN-γ and by inducing anti-inflammatory IL-4 production (Zanone et al., 2010). These data stimulate further studies on MSC immunemodulation in diabetes and open a perspective for immune-intervention strategies. The mechanisms of MSC interaction with the immune system cells are still controversial (van Laar & Tyndall, 2006; Abdi et al., 2008), and include reduction of the expression of lymphocyte activation markers, change of the cytokine profile of dendritic cells, naive and activated T cells and NK cells to an anti-inflammatory phenotype, and increase of the regulatory T cell population (Aggarwal & Pittenger, 2005; Le Blanc et al., 2004). In the reported study in type 1 diabetes, MSC induced in peripheral blood mononuclear cells (PBMC) of responder patients IL-4 producing cells and IL-4 secretion, suggesting a possible switch to an anti-inflammatory Th2 signalling of T cells (Zanone et al., 2010). Increased IL-4 secretion has been shown in studies of MSC cocultured with subpopulations of PHA-stimulated immune cells (Aggarwal & Pittenger, 2005) but not in studies of T cells activated by encephalitogenic peptide (Zappia et al., 2005). Lymphocyte activation is extremely complex and it is likely that several mechanisms are involved in the MSC-mediated immunesuppression and that the specific factors may depend on the lymphocyte population tested, the stimulus used, the timing of analysis and the context of the immune disease.

Further, inhibition of PGE_2 production abrogated the MSC-mediated IFN-γ suppression, indicating that PGE_2 secretion plays a key role in MSC-mediated immune effects, and the contact between MSC and PBMC enhances the production of prostaglandin E2 (Zanone et al., 2010). This observation suggests the requirement of both soluble factors and cell-contact in line with the interpretation that the immunemodulatory effects of MSC might require an initial cell-contact phase (Krampera et al., 2003). Nevertheless, the requirement of cell contact for MSC to operate their inhibition is a controversial issue, and the results reported in the literature may depend on the species and the type of stimulus.

Other studies in murine diabetic models on the regenerative capabilities of MSC, showed that injection of MSC into immunodeficient diabetic NOD/SCID mice resulted in the selective homing of MSC to pancreatic islets and in an increased number of pancreatic islets and functioning β cells (R.H. Lee et al., 2006). Further, MSC can be influenced to differentiate into cells with properties of the β cell phenotype, becoming more efficient after transplantation in mice (M. Zhao et al., 2008). Indeed, genetically modified MSC by recombinant Pdx-1 adenovirus or by non-virus gene transfection, were able to express insulin sufficient to reduce blood glucose in the streptozocin mouse model of diabetes (Karnieli et al., 2007; M. Zhao et al., 2008). More recently, allogenic MSC obtained from diabetes-prone as well as -resistant mice and injected into NOD mice, have been shown to delay the onset of diabetes or to reverse hyperglycemia (Fiorina et al., 2009). This study indicates that the beneficial effects observed could also be ascribed to the immunemodulatory capacities of MSC, as for other studies focusing on MSC-induced repair of cell injury (Abdi et al., 2008; Morigi et al., 2004; Duffield et al., 2005).

Studies on *ex vivo* expanded MSC to improve the outcome of allogenic transplantation and of acute graft-versus-host disease paved the way for the clinical use of MSC also in

autoimmune diseases. In experimental autoimmune encephalomyelitis, animal model of multiple sclerosis mediated by autoreactive T cells, injected MSC home to lymphoid organs where they cluster around T cells, and ameliorate the disease onset (Gerdoni et al., 2007). In this setting, MSC induce peripheral tolerance, impairing both the cellular and humoral arm of the encephalitogenic immune response, without evidence of transdifferentiation into neural cells (Zappia et al., 2005; Gerdoni et al., 2007). Furthermore, in a murine model of rheumatoid arthritis MSC have been demonstrated to exert an immunemodulatory effect by educating antigen-specific regulatory T cells (Augello et al., 2007). MSCs are also able to inhibit autoreactive T and B cells in experimental models of systemic lupus erythematosus (Deng et al., 2005). Overall, studies on murine models of type 1 diabetes (Fiorina et al., 2009; Madec et al., 2009) and multiple sclerosis (Zappia et al., 2005) as well as non-autoimmune diseases (Herrera et al., 2007) indicate that a key feature of MSC is their ability to selectively migrate into sites of injury, where they are likely to interact with activated T cells. Diabetogenic T cells are generated in pancreatic lymph nodes where they are introduced to antigens by dendritic cells. The preferential homing of MSC to pancreatic lympho nodes (Fiorina et al., 2009; Madec et al., 2009), supports the hypothesis that these cells could directly suppress autoreactive T cells *in vivo* within the pancreatic environment. Further, the desired therapeutic effects could be achieved by modulation of chemokines/receptors to promote the homing of MSC to specific anatomical sites (Sackstein et al., 2008). The first report on transplantation of human allogenic MSC, into a patient with autoimmune systemic sclerosis, indicates its safety and, notably, striking efficacy by selective immunesuppression and regeneration of impaired endothelial progenitors (Christopeit et al., 2008). Thus, results on the use of MSC infusion for treatment of severe graft-versus-host disease or autoimmune diseases (Lazarus et al., 2005; Fouillard et al., 2003; Le Blanc et al., 2004; Ringden et al., 2006; Christopeit et al., 2008) suggest a potential use in patients at risk of type 1 diabetes or at disease onset, to preserve or reduce loss of ß cells (Abdi et al., 2008; Staeva-Vieira et al., 2007).

7. MSC treatment of complications of diabetes mellitus

The ability of MSC to differentiate into tissue of mesodermal origin makes them attractive also as therapeutic agents for a number of complications of diabetes, including cardiomyopathy, nephropathy, polyneuropathy and diabetic wounds. MSC have been shown to differentiate into several cell types, including cardiomyocytes, endothelial cells, neurons, hepatocytes, epithelial cells and adipocytes, characteristics coupled with capacity of self renewal.

Chronic hyperglycemia is responsible for myocardial remodelling leading to ventricular dysfunction with hypertrophy and apoptosis of cardiomyocytes, microcirculatory defects, altered extracellular matrix and matrix metalloproteinasis (Jesmin et al., 2003). MSC can induce myogenesis and angiogenesis by different mitogenic, angiogenic and antiapoptotic factors, such as VEGF, IGF-1 and HGF (Zhang et al., 2008). In a diabetic rat model, intravenous MSC have been shown to improve cardiac function, potentially by differentiation into cardiomyocytes and improvement of myogenesis and angiogenesis. Metalloproteinase activity was also modulated, leading to increased arteriolar density and decreased collagen volume. Cardioprotection is probably more mediated by release of paracrine factors by MSC. These include VEGF, HGF, Bcl-2, Hsp20, activation of Akt

(Wang et al., 2009), which can affect remodelling, repair and neovascularization. Intravenous autologous MSC in post-infarction patients have indeed been shown to reduce episodes of ventricular tachycardia and to increase ventricular ejection fraction (R.H. Lee et al., 2010).
Diabetic limb ischemia could also be improved by MSC-derived pro-angiogenic factors (Comerota et al., 2010).
MSC have also been used for treatment of diabetic nephropathy in NOD/SCID and streptozocin C57B1/6 mice (R.H. Lee et al., 2006). The injected cells engraft in damaged kidneys, potentially differentiate into renal cells and endothelial cells and can regulate the immune response. This resulted in improved kidney function and regeneration of glomerular structure. MSC, however, were unable to proliferate; therefore, it is conceivable that MSC contribute to the repair by releasing paracrine factors that promote neovasculatization and limit cytotoxic injury.
As for diabetic polyneuropathy, in diabetic rats, intramuscular injection of MSC led to increased ratio of capillaries to muscle fibers, improvement of hyperalgesia and function of neural fibers. MSC settled in the gap between muscle fibers at the transplanted site, and produced VEGF and basic bFGF, without differentiating into neural cells (Shibata et al., 2008).
By releasing paracrine factors and by differentiation into photoreceptor and glial-like cells in the retina, transplanted MSC have also been shown to improve the integrity of the blood-retinal barrier, ameliorating diabetic retinopathy in streptozocin diabetic rats (Z. Yang et al., 2010).
Prolonged and uncompleted wound healing can complicate the diabetic condition. Injection of MSC in animal models of diabetes improved wound healing, with increase of collagen levels and of wound-breaking strength, together with increased levels of TFGβ, KGF, EGF, PDGF, VEGF, all involved in repair (Wu et al., 2007). Besides these paracrine effects MSC were shown to differentiate and regenerate damaged epithelium.
Limitation of the potential therapeutic use of MSC also for diabetic chronic complications are, at present, mainly the poor engraftment and the limited differentiation under *in vivo* conditions, together with the potential differentiation into unwanted mesenchymal lineages.

8. Conclusion

A number of issues should be addressed before a cell based therapy may come to a clinical setting. The first challenge is to define which kind of cells are more suitable for β cell substitution. This implies to develop efficient strategies of stem cell differentiation that lead to cells that produce and secrete insulin in physiological amounts under the control of glycemia. Moreover, safety of a cell based therapy remains a critical point, as any precursor or stem cell types might induce tumor formation. Whether the achievement of fully differentiated cells would reduce this risk remains to be proved. Another relevant point is to define the strategies that allow an immune modulation to avoid the recurrence of autoimmune destruction of newly formed β cells.

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10. References

Abdi, R., Fiorina, P., Adra, C.N., Atkinson, M. & Sayegh, M.H. (2008). Immunomodulation by mesenchymal stem cells: a potential therapeutic strategy for type 1 diabetes. *Diabetes*, Vol. 57, No. 7, (July 2008), pp. 1759-1767, ISSN 0012-1797.

Aggarwal, S. & Pittenger, M.F. (2005). Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood,* Vol. 105, No. 4, (February 2005), pp. 1815-1822, ISSN 0006-4971

Alipio, Z., Liao, W., Roemer, E.J., Waner, M., Fink, L.M., Ward, D.C. & Ma, Y. (2010). Reversal of hyperglycemia in diabetic Augello mouse models using induced-pluripotent stem (iPS)-derived pancreatic beta-like cells. *Proc Natl Acad Sci U S A*, Vol. 107, No. 30, (July 2010), pp. 13426-13431, ISSN 0027-8424

Augello, A., Tasso.,R., Negrini, S.M., Cancedda, R. & Pennesi, G. (2007). Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis. *Arthritis Rheum*, Vol. 56, No. 4, (April 2007), pp. 1175-1186, ISSN 00043591

Baeyens, L., De Breuck, S., Lardon, J., Mfopou, J.K., Rooman, I. & Bouwens, L. (2005). In vitro generation of insulin-producing beta cells from adult exocrine pancreatic cells. *Diabetologia*, Vol. 48, No. 1, (January 2005), pp. 49-57, ISSN 0012-186X

Bernal-Mizrachi, E., Fatrai, S., Johnson, J.D., Ohsugi, M., Otani, K., Han, Z., Polonsky, K.S. & Permutt, M.A. (2004). Defective insulin secretion and increased susceptibility to experimental diabetes are induced by reduced Akt activity in pancreatic islet beta cells. *J Clin Invest*, Vol. 114, No. 7, (October 2004), pp. 928-936, ISSN 0021-9738

Biancone, L. & Ricordi, C. (2002). Pancreatic islet transplantation: an update. *Cell Transplant,* Vol. 11, No. 4, pp. 309-311, ISSN 0963-6897

Bonner-Weir, S., Toschi, E., Inada, A., Reitz, P., Fonseca, S.Y., Aye, T. & Sharma, A. (2004). The pancreatic ductal epithelium serves as a potential pool of progenitor cells. *Pediatr Diabetes,* Vol. 5, Suppl 2, pp. 16-22, ISSN 1399-543X

Booth, C. & Potten, C.S. (2000). Gut instincts: thoughts on intestinal epithelial stem cells. *J Clin Invest,* Vol. 105, No. 11, (June 2000), pp. 1493-1499, ISSN 0021-9738

Bouvens, L. (2006). Beta cell regeneration. *Curr Diabetes Rev,* Vol. 2, pp. 3-9 ISSN 1573-3998

Brissova, M., Fowler, M., Wiebe, P., Shostak, A., Shiota, M., Radhika, A., Lin, P.C., Gannon, M. & Powers, A.C. (2004). Intraislet endothelial cells contribute to revascularization of transplanted pancreatic islets. *Diabetes*, Vol. 53, No. 5, (May 2004), pp. 1318-1325, ISSN 0012-1797

Brolén, G.K., Heins, N., Edsbagge, J. & Semb, H. (2005). Signals from the embryonic mouse pancreas induce differentiation of human embryonic stem cells into insulin-producing beta-cell-like cells. *Diabetes*, Vol. 54, No. 10, (October 2005), pp. 2867-2874, ISSN 0012-1797

Bussolati, B., Bruno, S., Grange, C., Buttiglieri, S., Deregibus, M.C., Cantino, D. & Camussi, G. (2005). Isolation of renal progenitor cells from adult human kidney. *Am J Pathol*, Vol. 166, No. 2, (February 2005), pp. 545-555, ISSN 0002-9440

Calafiore, R., Basta, G., Luca, G., Lemmi, A., Montanucci, M.P., Calabrese, G., Racanicchi, L., Mancuso, F. & Brunetti, P. (2006). Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: first two cases. *Diabetes Care*, Vol. 29, No. 1, (January 2006), pp. 137-138, ISSN 0149-5992

Cantaluppi, V., Biancone, L., Romanazzi, G.M., Figliolini, F., Beltramo, S., Ninniri, M.S., Galimi, F., Romagnoli, R., Franchello, A., Salizzoni, M., Perin, P.C., Ricordi, C., Segoloni, G.P. & Camussi, G. (2006). Antiangiogenic and immunomodulatory effects of rapamycin on islet endothelium: relevance for islet transplantation. *Am J Transplant*, Vol. 6, No. 11, (November 2006), pp. 2601-2611, ISSN 1600-6135

Cantaluppi, V., Bruno, S. & Camussi, G. (2008). Pancreatic ductal transdifferentiation for β-cell neogenesis. *Expert Opin Ther Patents*, Vol. 18, No. 8, pp. 1-5, ISSN 1354-3776

Carlsson, P.O., Andersson, A. & Jansson, L. (1998). Influence of age, hyperglycemia, leptin, and NPY on islet blood flow in obese-hyperglycemic mice. *Am J Physiol*, Vol. 275, No. 4 Pt 1, (October 1998), pp. E594-601, ISSN 0002-9513

Carlsson, P.O., Flodström, M. & Sandler, S. (2000). Islet blood flow in multiple low dose streptozotocin-treated wild-type and inducible nitric oxide synthase-deficient mice. *Endocrinology*, Vol. 141, No. 8, (August 2000), pp. 2752-2757, ISSN 0013-7227

Chao, K.C., Chao, K.F., Fu, Y.S. & Liu, S.H. (2008). Islet-like clusters derived from mesenchymal stem cells in Wharton's Jelly of the human umbilical cord for transplantation to control type 1 diabetes. *PLoS One*, Vol. 3, No. 1, (January 2008), pp. e1451, ISSN 1932-6203

Christopeit, M., Schendel, M., Föll, J., Müller, L.P., Keysser, G. & Behre, G. (2008). Marked improvement of severe progressive systemic sclerosis after transplantation of mesenchymal stem cells from an allogeneic haploidentical-related donor mediated by ligation of CD137L. *Leukemia*, Vol. 22, No. 5, (May 2008), pp. 1062-1064, ISSN 0887-6924

Cnop, M., Hughes, S.J., Igoillo-Esteve, M., Hoppa, M.B., Sayyed, F., van de Laar, L., Gunter, J.H., de Koning, E.J., Walls, G.V., Gray, D.W., Johnson, P.R., Hansen, B.C., Morris, J.F., Pipeleers-Marichal, M., Cnop, I. & Clark, A. (2010). The long lifespan and low turnover of human islet beta cells estimated by mathematical modelling of lipofuscin accumulation. *Diabetologia*, Vol. 53, No. 2, (February 2010), pp. 321-330, ISSN 0012-186X

Colvin, G.A., Lambert, J.F., Moore, B.E., Carlson, J.E., Dooner, M.S., Abedi, M., Cerny, J. & Quesenberry, P.J. (2004). Intrinsic hematopoietic stem cell/progenitor plasticity: Inversions. *J Cell Physiol*, Vol. 199, No. 1, (April 2004), pp. 20-31, ISSN 0021-9541

Colvin, G.A., Dooner, M.S., Dooner, G.J., Sanchez-Guijo, F.M., Demers, D.A., Abedi, M., Ramanathan, M., Chung, S., Pascual, S. & Quesenberry, P.J. (2007). Stem cell continuum: directed differentiation hotspots. *Exp Hematol*, Vol. 35, No. 1, (January 2007), pp. 96-107, ISSN 0301-472X

Comerota, A.J., Link, A., Douville, J. & Burchardt, E.R. (2010). Upper extremity ischemia treated with tissue repair cells from adult bone marrow. *J Vasc Surg*, Vol. 52, No. 3, (September 2010), pp. 723-729, ISSN 0741-5214

Contreras, J.L., Smyth, C.A., Eckstein, C., Bilbao, G., Thompson, J.A., Young, C.J. & Eckhoff, D.E. (2003). Peripheral mobilization of recipient bone marrow-derived endothelial progenitor cells enhances pancreatic islet revascularization and engraftment after intraportal transplantation. *Surgery,* Vol. 134, No. 2, (August 2003), pp. 390-398, ISSN 0039-6060

Cotsarelis, G., Sun, T.T. & Lavker, R.M. (1990). Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. *Cell*, Vol. 61, No. 7, (June 1990), pp. 1329-1337, ISSN 0092-8674

Cronin, K.J., Messina, A., Knight, K.R., Cooper-White, J.J., Stevens, G.W., Penington, A.J. & Morrison, W.A. (2004). New murine model of spontaneous autologous tissue engineering, combining an arteriovenous pedicle with matrix materials. *Plast Reconstr Surg*, Vol. 113, No. 1, (January 2004), pp. 260-269, ISSN 0032-1052

D'Amour, K.A., Agulnick, A.D., Eliazer, S., Kelly, O.G., Kroon, E. & Baetge, E.E. (2005). Efficient differentiation of human embryonic stem cells to definitive endoderm. *Nat Biotechnol*, Vol. 23, No. 12, (December 2005), pp. 1534-1541, ISSN 1087-0156

da Silva Meirelles, L., Chagastelles, P.C. & Nardi, N.B. (2006). Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci*, Vol. 119, No. 11, (June 2006), pp. 2204-2213, ISSN 0021-9533

da Silva Meirelles, L., Caplan, A.I. & Nardi, N.B. (2008). In search of the in vivo identity of mesenchymal stem cells. *Stem Cells*, Vol. 26, No. 9, (September 2008), pp. 2287-2299, ISSN 1066-5099

Datta, S.R., Brunet, A. & Greenberg, M.E. (1999). Cellular survival: a play in three Akts. *Genes Dev*, Vol. 13, No. 22, (November 1999), pp. 2905-2927, ISSN 0890-9369

Deng, W., Han, Q., Liao, L., You, S., Deng, H. & Zhao, R.C. (2005). Effects of allogeneic bone marrow-derived mesenchymal stem cells on T and B lymphocytes from BXSB mice. *DNA Cell Biol*, Vol. 24, No. 7, (July 2005), pp. 458-463, ISSN 1044-5498

Denner, L., Bodenburg, Y., Zhao, J.G., Howe, M., Cappo, J., Tilton, R.G., Copland, J.A., Forraz, N., McGuckin, C. & Urban, R. (2007). Directed engineering of umbilical cord blood stem cells to produce C-peptide and insulin. *Cell Prolif*, Vol. 40, No. 3, (June 2007), pp. 367-380, ISSN 0960-7722

Deregibus, M.C., Tetta, C. & Camussi, G. (2010). The dynamic stem cell microenvironment is orchestrated by microvesicle-mediated transfer of genetic information. *Histol Histopathol*, Vol. 25, No. 3, (March 2010), pp. 397-404, ISSN 0213-3911

Doetsch, F., Caillé, I., Lim, D.A., García-Verdugo, J.M. & Alvarez-Buylla, A. (1999). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell*, Vol. 97, No. 6, (June 1999), pp. 703-716, ISSN 0092-8674

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F., Krause, D., Deans, R., Keating, A., Prockop, D.J. & Horwitz, E. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*, Vol. 8, No. 4, pp. 315-317, ISSN 1465-3249

Donath, M.Y. & Halban, P.A. (2004). Decreased beta-cell mass in diabetes: significance, mechanisms and therapeutic implications. *Diabetologia*, Vol. 47, No. 3, (March 2004), pp. 581-589, ISSN 0012-186X

Dor, Y., Brown, J., Martinez, O.I. & Melton, D.A. (2004). Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature*, Vol. 429, No. 6987, (May 2004), pp. 41-46, ISSN 0028-0836

Duffield, J.S., Park, K.M., Hsiao, L.L., Kelley, V.R., Scadden, D.T., Ichimura, T. & Bonventre, J.V. (2005). Restoration of tubular epithelial cells during repair of the postischemic kidney occurs independently of bone marrow-derived stem cells. *J Clin Invest*, Vol. 115, No. 7, (July 2005), pp. 1743-1755, ISSN 0021-9738

Elghazi, L., Balcazar, N. & Bernal-Mizrachi, E. (2006). Emerging role of protein kinase B/Akt signaling in pancreatic beta-cell mass and function. *Int J Biochem Cell Biol*, Vol. 38, No. 2, (February 2006), pp. 157-163, ISSN 1357-2725

Elliott, R.B., Terán, L. & White, D.J. (2005). Xenotransplantation of porcine neonatal islets of Langerhans and Sertoli cells: a 4-year study. *Eur J Endocrinol*, Vol. 153, No. 3, (September 2005), pp. 419-427, ISSN 0804-4643

Elliott, R.B., Escobar, L., Tan, P.L., Muzina, M., Zwain, S. & Buchanan, C. (2007). Live encapsulated porcine islets from a type 1 diabetic patient 9.5 yr after xenotransplantation. *Xenotransplantation*, Vol. 14, No. 2, (March 2007), pp. 157-161, ISSN 0908-665X

Fan, Y., Rudert, W.A., Grupillo, M., He, J., Sisino, G. & Trucco, M. (2009). Thymus-specific deletion of insulin induces autoimmune diabetes. *EMBO J*, Vol. 28, No. 18, (September 2009), pp. 2812-24, ISSN 0261-4189

Faradji, R.N., Tharavanij, T., Messinger, S., Froud, T., Pileggi, A., Monroy, K., Mineo, D., Baidal, D.A., Cure, P., Ponte, G., Mendez, A.J., Selvaggi, G., Ricordi, C. & Alejandro, R. (2008). Long-term insulin independence and improvement in insulin secretion after supplemental islet infusion under exenatide and etanercept. *Transplantation*, Vol. 86, No. 12, (December 2008), pp. 1658-65, ISSN 0041-1337

Favaro, E., Miceli, I., Bussolanti, B., Schmitt-Ney, M., Cavallo Perin, P., Camusi, G. & Zanone, M.M. (2008). Hyperglycemia induces apoptosis of human pancreatic islet endothelial cells: effects of pravastatin on the Akt survival pathway. *Am J Pathol*, Vol. 173, No. 2, (August 2008), pp. 442-450, ISSN 0002-9440

Fiorina, P., Jurewicz, M., Augello, A., Vergani, A., Dada, S., La Rosa, S., Selig, M., Godwin, J., Law, K., Placidi, C., Smith, R.N., Capella, C., Rodig, S., Adra, C.N., Atkinson, M., Sayegh, M.H. & Abdi, R. (2009). Immunomodulatory function of bone marrow-derived mesenchymal stem cells in experimental autoimmune type 1 diabetes. *J Immunol*, Vol. 183, No. 2, (July 2009), pp. 993-1004, ISSN 0022-1767

Fouillard, L., Bensidhoum, M., Bories, D., Bonte, H., Lopez, M., Moseley, A.M., Smith, A., Lesage, S., Beaujean, F., Thierry, D., Gourmelon, P., Najman, A. & Gorin, N.C. (2003). Engraftment of allogeneic mesenchymal stem cells in the bone marrow of a patient with severe idiopathic aplastic anemia improves stroma. *Leukemia*, Vol. 17, No. 2, (February 2003), pp. 474-476, ISSN 0887-6924

Froud, T., Faradji, R.N., Pileggi, A., Messinger, S., Baidal, D.A., Ponte, G.M., Cure, P.E., Monroy, K., Mendez, A., Selvaggi, G., Ricordi, C. & Alejandro, R. (2008). The use of exenatide in islet transplant recipients with chronic allograft dysfunction: safety, efficacy, and metabolic effects. *Transplantation*, Vol. 86, No. 1, (July 2008), pp. 36-45, ISSN 0041-1337

Gerdoni, E., Gallo, B., Casazza, S., Musio, S., Bonanni, I., Pedemonte, E., Mantegazza, R., Frassoni, F., Mancardi, G., Pedotti, R. & Uccelli, A. (2007). Mesenchymal stem cells effectively modulate pathogenic immune response in experimental autoimmune encephalomyelitis. *Ann Neurol*, Vol. 61, No. 3, (March 2007), pp. 219-227, ISSN 0364-5134

Gonez, L.J. & Knight, K.R. (2010). Cell therapy for diabetes: stem cells, progenitors or beta-cell replication? *Mol Cell Endocrinol*, Vol. 323, No. 1, (July 2010), pp. 55-61, ISSN 0303-7207

Gottlieb, P.A., Quinlan, S., Krause-Steinrauf, H., Greenbaum, C.J., Wilson, D.M., Rodriguez, H., Schatz, D.A., Moran, A.M., Lachin, J.M. & Skyler, J.S. (2010). Type 1 Diabetes TrialNet MMF/DZB Study Group. Failure to preserve beta-cell function with mycophenolate mofetil and daclizumab combined therapy in patients with new-

onset type 1 diabetes. *Diabetes Care*, Vol. 33, No. 4, (April 2010), pp. 826-832, ISSN 0149-5992

Hafiz, M.M., Faradji, R.N., Froud, T., Pileggi, A., Baidal, D.A., Cure, P., Ponte, G., Poggioli, R., Cornejo, A., Messinger, S., Ricordi, C. & Alejandro, R. (2005). Immunosuppression and procedure-related complications in 26 patients with type 1 diabetes mellitus receiving allogeneic islet cell transplantation. *Transplantation*, Vol. 80, No. 12, (December 2005), pp. 1718-1728, ISSN 0041-1337

Haller, M.J., Viener, H.L., Brusko, T., Wasserfall, C., McGrail, K., Staba, S. & Cogle, C., Atkinson, M. & Schatz, D.A. (2007). Insulin requirements, HbA1c, and stimulated C-peptide following autologous umbilical cord blood transfusion in children with T1D. *Diabetes*, Vol. 56, No. A82 Suppl. 1 (June 2007), ISSN 0012-1797

He, X.C., Zhang, J., Tong, W.G., Tawfik, O., Ross, J., Scoville, D.H., Tian, Q., Zeng, X., He, X., Wiedemann, L.M., Mishina, Y. & Li, L. (2004). BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-beta-catenin signaling. *Nat Genet*, Vol. 36, No. 10, (October 2004), pp. 1117-1121, ISSN 1061-4036

Herold, K.C., Hagopian, W., Auger, J.A., Poumian-Ruiz, E., Taylor, L., Donaldson, D., Gitelman, S.E., Harlan, D.M., Xu, D., Zivin, R.A. & Bluestone J.A. (2002). Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med*, Vol. 346, No. 22, (May 2002), pp. 1692-1698, ISSN 0028-4793

Herrera, M.B., Bruno, S., Buttiglieri, S., Tetta, C., Gatti, S., Deregibus, M.C., Bussolati, B. & Camussi, G. (2006). Isolation and characterization of a stem cell population from adult human liver. *Stem Cells*, Vol. 24, No. 12, (December 2006), pp. 2840-2850, ISSN 1066-5099

Herrera, M.B., Bussolati, B., Bruno, S., Morando, L., Mauriello-Romanazzi, G., Sanavio, F., Stamenkovic, I., Biancone, L. & Camussi, G. (2007). Exogenous mesenchymal stem cells localize to the kidney by means of CD44 following acute tubular injury. *Kidney Int*, Vol. 72, No. 4, (August 2007), pp. 430-441, ISSN 0085-2538

Hussey, A.J., Winardi, M., Han, X.L., Thomas, G.P., Penington, A.J., Morrison, W.A., Knight, K.R. & Feeney, S.J. (2009). Seeding of pancreatic islets into prevascularized tissue engineering chambers. *Tissue Eng Part A*, Vol. 15, No. 12, (December 2009), pp. 3823-3833, ISSN 2152-4947

Ito, T., Itakura, S., Todorov, I., Rawson, J., Asari, S., Shintaku, J., Nair, I., Ferreri, K., Kandeel, F. & Mullen, Y. (2010). Mesenchymal stem cell and islet co-transplantation promotes graft revascularization and function. *Transplantation*, Vol. 89, No. 12, (June 2010), pp. 1438-1445, ISSN 0041-1337

Jackson, K.A., Mi, T. & Goodell, M.A. (1999).Hematopoietic potential of stem cells isolated from murine skeletal muscle. *Proc Natl Acad Sci U S A*, Vol. 96, No. 25, (December 1999), pp. 14482-14486, ISSN 0027-8424

Jesmin, S., Sakuma, I., Hattori, Y. & Kitabatake, A. (2003). Role of angiotensin II in altered expression of molecules responsible for coronary matrix remodeling in insulin-resistant diabetic rats. *Arterioscler Thromb Vasc Biol*, Vol. 23, No. 11, (November 2003), pp. 2021-2026, ISSN 1079-5642

Jiang, J., Au, M., Lu, K., Eshpeter, A., Korbutt, G., Fisk, G. & Majumdar, A.S. (2007). Generation of insulin-producing islet-like clusters from human embryonic stem cells. *Stem Cells*, Vol. 25, No. 8, (August 2007), pp. 1940-1953, ISSN 1066-5099

Jiang, Y., Jahagirdar, B.N., Reinhardt, R.L., Schwartz, R.E., Keene, C.D., Ortiz-Gonzalez, X.R., Reyes, M., Lenvik, T., Lund, T., Blackstad, M., Du, J., Aldrich, S., Lisberg, A., Low, W.C., Largaespada, D.A. & Verfaillie, C.M. (2002). Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*, Vol. 418, No. 6893, (July 2002), pp. 41-49, ISSN 0028-0836

Jones, D.L. & Wagers, A.J. (2008). No place like home: anatomy and function of the stem cell niche. *Nat Rev Mol Cell Biol*, Vol. 9, No. 1, (January 2008), pp. 11-21, ISSN 1083-8791

Juhl, K., Bonner-Weir, S. & Sharma, A. (2010). Regenerating pancreatic beta-cells: plasticity of adult pancreatic cells and the feasibility of in-vivo neogenesis. *Curr Opin Organ Transplant*, Vol. 15, No. 1,(February 2010), pp. 79-85, ISSN 1531-7013

Jung, F., Haendeler, J., Goebel, C., Zeiher, A.M. & Dimmeler, S. (2000). Growth factor-induced phosphoinositide 3-OH kinase/Akt phosphorylation in smooth muscle cells: induction of cell proliferation and inhibition of cell death. *Cardiovasc Res*, Vol. 48, No. 1, (October 2000), pp. 148-57, ISSN 0008-6363

Karnieli, O., Izhar-Prato, Y., Bulvik, S. & Efrat, S. (2007). Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. *Stem Cells*, Vol. 25, No. 11, (November 2007), pp. 2837-2844, ISSN 1066-5099

Keymeulen, B., Vandemeulebroucke, E., Ziegler, A.G., Mathieu, C., Kaufman, L., Hale, G., Gorus, F., Goldman, M., Walter, M., Candon, S., Schandene, L., Crenier, L., De Block, C., Seigneurin, J.M., De Pauw, P., Pierard, D., Weets, I., Rebello, P., Bird, P., Berrie, E., Frewin, M., Waldmann, H., Bach, J.F., Pipeleers, D. & Chatenoud, L. (2005). Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med*, Vol. 352, No. 25, (June 2005), pp. 2598-2608, ISSN 0028-4793

Kiel, M.J., Yilmaz, O.H., Iwashita, T., Yilmaz, O.H., Terhorst, C. & Morrison, S.J. (2005). SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell*, Vol. 121, No. 7, (July 2005), pp. 1109-1121, ISSN 0092-8674

Kojima, H., Fujimiya, M., Matsumura, K., Younan, P., Imaeda, H., Maeda, M. & Chan, L. (2003). NeuroD-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. *Nat Med*, Vol. 9, No. 5, (May 2003), pp. 596-603, ISSN 1078-8956

Koopmans, M., Kremer Hovinga, I.C., Baelde, H.J., de Heer, E., Bruijn, J.A. & Bajema, I.M. Endothelial chimerism in transplantation: Looking for needles in a haystack. *Transplantation*, Vol. 82 Suppl. 1, pp. S25-S29, ISSN 0041-1337

Krampera, M., Glennie, S., Dyson, J., Scott, D., Laylor R, Simpson, E. & Dazzi, F. (2003). Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. *Blood*, Vol. 101, No. 9, (May 2003), pp. 3722-3729, ISSN 0006-4971

Kröncke, K.D., Rodriguez, M.L., Kolb, H. & Kolb-Bachofen, V. (1993). Cytotoxicity of activated rat macrophages against syngeneic islet cells is arginine-dependent, correlates with citrulline and nitrite concentrations and is identical to lysis by the nitric oxide donor nitroprusside. *Diabetologia*, Vol. 36, No. 1, (January 1993), pp. 17-24, ISSN 0012-186X

Kroon, E., Martinson, L.A., Kadoya, K., Bang, A.G., Kelly, O.G., Eliazer, S., Young, H., Richardson, M., Smart, N.G., Cunningham, J., Agulnick, A.D., D'Amour, K.A., Carpenter, M.K. & Baetge, E.E. (2008). Pancreatic endoderm derived from human

embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. *Nat Biotechnol*, Vol. 26, No. 4, (April 2008), pp. 443-452, ISSN 1087-0156

Lazarus, H.M., Koc, O.N., Devine, S.M., Curtin, P., Maziarz, R.T., Holland, H.K., Shpall, E.J., McCarthy, P., Atkinson, K., Cooper, B.W., Gerson, S.L., Laughlin, M.J., Loberiza, F.R. Jr, Moseley, A.B. & Bacigalupo, A. (2005). Cotransplantation of HLA-identical sibling culture-expanded mesenchymal stem cells and hematopoietic stem cells in hematologic malignancy patients. *Biol Blood Marrow Transplant*, Vol. 11, No. 5, (May 2005), pp. 389-398, ISSN 1083-8791

Le Blanc, K., Rasmusson, I., Sundberg, B., Götherström, C., Hassan, M., Uzunel, M. & Ringdén, O. (2004). Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet*, Vol. 363, No. 9419, (May 2004), pp. 1439-1441, ISSN 0140-6736

Le Blanc, K., Rasmusson, I., Götherström, C., Seidel, C., Sundberg, B., Sundin, M., Rosendahl, K., Tammik, C. & Ringdén, O. (2004). Mesenchymal stem cells inhibit the expression of CD25 (interleukin-2 receptor) and CD38 on phytohaemagglutinin-activated lymphocytes. *Scand J Immunol*, Vol. 60, No. 3, (September 2004), pp. 307-315, ISSN 0300-9475

Le Blanc, K. & Pittenger M. (2005). Mesenchymal stem cells: progress toward promise. *Cytotherapy*, Vol. 7, No. 1, pp. 36-45, ISSN 1465-3249

Lee, J.S., Hong, J.M., Moon, G.J., Lee, P.H., Ahn, Y.H. & Bang, O.Y.; STARTING collaborators. (2010). A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. *Stem Cells*, Vol. 28, No. 6, (June 2010), pp. 1099-1106, ISSN 1066-5099

Lee, R.H., Seo, M.J., Reger, R.L., Spees, J.L., Pulin, A.A., Olson, S.D. & Prockop, D.J. (2006). Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice. *Proc Natl Acad Sci U S A*, Vol. 103, No. 46, (November 2006), pp. 17438-17443, ISSN 0027-8424

Lee, S.H., Hao, E., Savinov, A.Y., Geron, I., Strongin, A.Y. & Itkin-Ansari, P. (2009). Human beta-cell precursors mature into functional insulin-producing cells in an immunoisolation device: implications for diabetes cell therapies. *Transplantation*, Vol. 87, No. 7, (April 2009), pp. 983-991, ISSN 0041-1337

Lemaigre, F. & Zaret, K.S. (2004). Liver development update: new embryo models, cell lineage control, and morphogenesis. *Curr Opin Genet Dev*, Vol. 14, No. 5, (October 2004), pp. 582-590, ISSN 0959-437X

Li, L. & Xie, T. (2002). Stem cell niche: structure and function. *Annu Rev Cell Dev Biol*, Vol. 21, pp. 605-631, ISSN 1081-0706

Niemann, C. & Watt, F.M. (2002). Designer skin: lineage commitment in postnatal epidermis. *Trends Cell Biol*, Vol. 12, No. 4, (April 2002), pp. 185-192, ISSN 0962-8924

Li, L. & Xie T. (2005). Stem cell niche: structure and function. *Annu Rev Cell Dev Biol*, Vol. 21, pp. 605-631, ISSN 1081-0706

Li, W.C., Rukstalis, J.M., Nishimura, W., Tchipashvili, V., Habener, J.F., Sharma, A. & Bonner-Weir, S. (2010). Activation of pancreatic-duct-derived progenitor cells during pancreas regeneration in adult rats. *J Cell Sci*, Vol. 123, Pt16, (August 2010), pp. 2792-2802, ISSN 0021-9533

Li, X., Zhang, L., Meshinchi, S., Dias-Leme, C., Raffin, D., Johnson, J.D., Treutelaar, M.K. & Burant, C.F. (2006). Islet microvasculature in islet hyperplasia and failure in a

model of type 2 diabetes. *Diabetes*, Vol. 55, No. 11, (November 2006), pp. 2965-2973, ISSN 0012-1797

Li, Y., Zhang, R., Qiao, H., Zhang, H., Wang, Y., Yuan, H., Liu, Q., Liu, D., Chen, L. & Pei, X. (2007). Generation of insulin-producing cells from PDX-1 gene-modified human mesenchymal stem cells. *J Cell Physiol*, Vol. 211, No. 1, (April 2007), pp. 36-44, ISSN 0021-9541

Loweth, A.C., Williams, G.T., James, R.F., Scarpello, J.H. & Morgan, N.G. (1998). Human islets of Langerhans express Fas ligand and undergo apoptosis in response to interleukin-1beta and Fas ligation. *Diabetes*, Vol. 47, No. 5, (May 1998), pp. 727-732, ISSN 0012-1797

Ludvigsson, J., Faresjö, M., Hjorth, M., Axelsson, S., Chéramy, M., Pihl, M., Vaarala, O., Forsander, G., Ivarsson, S., Johansson, C., Lindh, A., Nilsson, N.O., Aman, J., Ortqvist, E., Zerhouni, P. & Casas, R. (2008). GAD treatment and insulin secretion in recent-onset type 1 diabetes. *N Engl J Med*, Vol. 359, No. 18, (October 2008), pp. 1909-1920, ISSN 0028-4793

Madec, A.M., Mallone, R., Afonso, G., Abou Mrad, E., Mesnier, A., Eljaafari, A. & Thivolet, C. (2009). Mesenchymal stem cells protect NOD mice from diabetes by inducing regulatory T cells. *Diabetologia*, Vol. 52, No. 7, (July 2009), pp. 1391-1399, ISSN 0012-186X

Maedler, K., Spinas, G.A., Lehmann, R., Sergeev, P., Weber, M., Fontana, A., Kaiser, N. & Donath, M.Y. (2001). Glucose induces beta-cell apoptosis via upregulation of the Fas receptor in human islets. *Diabetes*, Vol. 50, No. 8, (August 2001), pp. 1683-1690, ISSN 0012-1797

Maehr, R., Chen, S., Snitow, M., Ludwig, T., Yagasaki, L., Goland, R., Leibel, R.L. & Melton, D.A. (2009). Generation of pluripotent stem cells from patients with type 1 diabetes. *Proc Natl Acad Sci U S A*, Vol. 106, No. 37, (September 2009), pp. 15768-15773, ISSN 0027-8424

Mathews, V., Hanson, P.T., Ford, E., Fujita, J., Polonsky, K.S. & Graubert, T.A. (2004). Recruitment of bone marrow-derived endothelial cells to sites of pancreatic beta-cell injury. *Diabetes*, Vol. 53, No. 1, pp. 91-98, ISSN 0012-1797

Matveyenko, A.V., Georgia, S., Bhushan, A. & Butler, P.C. (2010). Inconsistent formation and nonfunction of insulin-positive cells from pancreatic endoderm derived from human embryonic stem cells in athymic nude rats. *Am J Physiol Endocrinol Metab*, Vol. 299, No. 5, (November 2010), pp. E713-E720, ISSN 0193-1849

McDevitt, H.O. & Unanue, E.R. (2008). Autoimmune diabetes mellitus--much progress, but challenges. *Adv Immunol*, Vol. 100, pp. 1-12, ISSN 0065-2776

Meier, J.J., Bhushan, A., Butler, A.E., Rizza, R.A. & Butler, P.C. (2005). Sustained beta cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? *Diabetologia,* Vol. 48, No. 11, (November 2005), pp. 2221-2228, ISSN 0012-186X

Meier, J.J., Ritzel, R.A., Maedler, K., Gurlo, T. & Butler, P.C. (2006). Increased vulnerability of newly forming beta cells to cytokine-induced cell death. *Diabetologia*, Vol. 49, No. 1, (January 2006), pp. 83-89, ISSN 0012-186X

Meier, J.J., Butler, A.E., Saisho, Y., Monchamp, T., Galasso, R., Bhushan, A., Rizza, R.A. & Butler, P.C. (2008). Beta-cell replication is the primary mechanism subserving the

postnatal expansion of beta-cell mass in humans. *Diabetes*, Vol. 57, No. 6, (June 2008), pp. 1584-1594, ISSN 0012-1797

Mian, R., Morrison, W.A., Hurley, J.V., Penington, A.J., Romeo, R., Tanaka, Y. & Knight, K.R. (2000). Formation of new tissue from an arteriovenous loop in the absence of added extracellular matrix. *Tissue Eng*, Vol. 6, No. 6, (December 2000), pp. 595-603, ISSN 1076-3279

Minami, K., Okuno, M., Miyawaki, K., Okumachi, A., Ishizaki, K., Oyama, K., Kawaguchi, M., Ishizuka, N., Iwanaga, T. & Seino, S. (2005). Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. *Proc Natl Acad Sci U S A*, Vol. 102, No. 42, (October 2005), pp. 15116-15121, ISSN 0027-8424

Mineo, D., Ricordi, C., Xu, X., Pileggi, A., Garcia-Morales, R., Khan, A., Baidal, D.A., Han, D., Monroy, K., Miller, J., Pugliese, A., Froud, T., Inverardi, L., Kenyon, N.S. & Alejandro, R. (2008). Combined islet and hematopoietic stem cell allotransplantation: a clinical pilot trial to induce chimerism and graft tolerance. *Am J Transplant*, Vol. 8, No. 6, (June 2008), pp. 1262-1274, ISSN 1600-6135

Mineo, D., Pileggi, A., Alejandro, R. & Ricordi, C. (2009). Point: steady progress and current challenges in clinical islet transplantation. *Diabetes Care*, Vol. 32, No. 8, (August 2009), pp. 1563-1569, ISSN 0149-5992

Mitsiadis, T.A., Barrandon, O., Rochat, A., Barrandon, Y. & De Bari, C. (2007). Stem cell niches in mammals. *Exp Cell Res*, Vol. 313, No. 16, (October 2007), pp. 3377-3385, ISSN 0014-4827

Miura, K., Okada, Y., Aoi, T., Okada, A., Takahashi, K., Okita, K., Nakagawa, M., Koyanagi, M., Tanabe, K., Ohnuki, M., Ogawa, D., Ikeda, E., Okano, H. & Yamanaka, S. (2009). Variation in the safety of induced luripotent stem cell lines. *Nat Biotechnol*, Vol. 27, No. 8, (August 2009), pp. 743-745, ISSN 1087-0156

Moore, K.A. & Lemischka, I.R. (2006). Stem cells and their niches. *Science*, Vol. 311, No. 5769, (March 2006), pp. 1880-1885, ISSN 0036-8075

Morigi, M., Imberti, B., Zoja, C., Corna, D., Tomasoni, S., Abbate, M., Rottoli, D., Angioletti, S., Benigni, A., Perico, N., Alison, M. & Remuzzi, G. (2004). Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure. *J Am Soc Nephrol*, Vol. 15, No. 7, (July 2004), pp. 1794-1804, ISSN 1046-6673

Nagaya, M., Katsuta, H., Kaneto, H., Bonner-Weir, S. & Weir, G.C. (2009). Adult mouse intrahepatic biliary epithelial cells induced in vitro to become insulin-producing cells. *J Endocrinol*, Vol. 201, No. 1, (April 2009), pp. 37-47, ISSN 0022-0795

Nir, T., Melton, D.A. & Dor, Y. (2007). Recovery from diabetes in mice by beta cell regeneration. *J Clin Invest*, Vol. 117, No. 9, (September 2007), pp. 2553-2561, ISSN 0021-9738

Opara, E.C., Mirmalek-Sani, S.H., Khanna, O., Moya, M.L. & Brey, E.M. (2010). Design of a bioartificial pancreas(+). *J Investig Med*, Vol. 58, No. 7, (October 2010), pp. 831-837, ISSN 1081-5589

Pileggi, A., Cobianchi, L., Inverardi, L. & Ricordi, C. (2006). Overcoming the challenges now limiting islet transplantation: a sequential, integrated approach. *Ann N Y Acad Sci*, Vol. 1079, (October 2006), pp. 383-398, ISSN 0077-8923

Quesenberry, P., Abedi, M., Dooner, M., Colvin, G., Sanchez-Guijo, F.M., Aliotta, J., Pimentel, J., Dooner, G., Greer, D., Demers, D., Keaney, P., Peterson, A., Luo, L. &

Foster, B. (2005). The marrow cell continuum: stochastic determinism. *Folia Histochem Cytobiol*, Vol. 43, No. 4, pp. 187-190, ISSN 1897-5631

Quesenberry, P.J., Colvin, G., Dooner, G., Dooner, M., Aliotta, J.M. & Johnson, K. (2007). The stem cell continuum: cell cycle, injury, and phenotype lability. *Ann N Y Acad Sci*, Vol. 1106, (June 2007), pp. 20-29, ISSN 0077-8923

Quesenberry, P.J. & Aliotta, J.M. (2008). The paradoxical dynamism of marrow stem cells: considerations of stem cells, niches, and microvesicles. *Stem Cell Rev*, Vol. 4, No. 3 (September 2008), pp. 137-147, ISSN 1550-8943

Raz, I., Elias, D., Avron, A., Tamir, M., Metzger, M. & Cohen, I.R. (2001). Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet*, Vol. 358, No. 9295, (November 2001), pp. 1749-1753, ISSN 1550-8943

Reynolds, B.A. & Weiss, S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science*, Vol. 255, No. 5052, (March 1992), pp. 1707-1710, ISSN 0036-8075

Ricordi, C. (2003). Islet transplantation: a brave new world. *Diabetes*, Vol. 52, pp. 1595-1603, ISSN 0012-1797

Ricordi, C. & Strom, T.B. (2004). Clinical islet transplantation: advances and immunological challenges. *Nat Rev Immunol*, Vol. 4, No. 4, (April 2004), pp. 259-268, ISSN 1474-1733

Ricordi, C. & Edlund, H. (2008). Toward a renewable source of pancreatic beta-cells. *Nat Biotechnol*, Vol. 26, No. 4, (April 2008), pp. 397-398, ISSN 1087-0156

Ringdén, O., Uzunel, M., Rasmusson, I., Remberger, M., Sundberg, B., Lönnies, H., Marschall, H.U., Dlugosz, A., Szakos, A., Hassan, Z., Omazic, B., Aschan, J., Barkholt, L. & Le Blanc, K. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. (2006). *Transplantation*, Vol. 81, No. 10, (May 2006), pp. 1390-1397, ISSN 0041-1337

Roncarolo, M.G. & Battaglia, M. (2007). Regulatory T-cell immunotherapy for tolerance to self antigens and alloantigens in humans. *Nat Rev Immunol*, Vol. 7, No. 8, (August 2007), pp. 585-598, ISSN 1474-1733

Rosenberg, L. (1995). In vivo cell transformation: neogenesis of beta cells from pancreatic ductal cells. *Cell Transplant*, Vol. 4, No. 4, (July-August 1995), pp. 371-383, ISSN 0963-6897

Rother, K.I., Spain, L.M., Wesley, R.A., Digon, B.J. 3rd, Baron, A., Chen, K., Nelson, P., Dosch, H.M., Palmer, J.P., Brooks-Worrell, B., Ring, M. & Harlan, D.M. (2009). Effects of exenatide alone and in combination with daclizumab on beta-cell function in long-standing type 1 diabetes. *Diabetes Care*, Vol. 32, No. 12, (December 2009), pp. 2251-2257, ISSN 0149-5992

Ryan, E.A., Paty, B.W., Senior, P.A., Bigam, D., Alfadhli, E., Kneteman, N.M., Lakey, J.R. & Shapiro, A.M. (2005). Five-year follow-up after clinical islet transplantation. *Diabetes*, Vol. 54, No. 7, (July 2005), pp. 2060-2069, ISSN 0012-1797

Sackstein, R., Merzaban, J.S., Cain, D.W., Dagia, N.M., Spencer, J.A., Lin, C.P. & Wohlgemuth, R. (2008). Ex vivo glycan engineering of CD44 programs human multipotent mesenchymal stromal cell trafficking to bone. *Nat Med*, Vol. 14, No. 2, (February 2008), pp. 181-187, ISSN 1078-8956

Saito, M., Iwawaki, T., Taya, C., Yonekawa, H., Noda, M., Inui, Y., Mekada, E., Kimata, Y., Tsuru, A. & Kohno, K. (2001). Diphtheria toxin receptor-mediated conditional and

targeted cell ablation in transgenic mice. *Nat Biotechnol*, Vol. 19, No. 8, (August 2001), pp. 746-750, ISSN 1087-0156

Sancho, E., Batlle, E. & Clevers, H. (2004). Signaling pathways in intestinal development and cancer. *Annu Rev Cell Dev Biol*, Vol. 20, pp. 695-723, ISSN 1081-0706

Sarvetnick, N., Krakowki, M.L. & Kritzik, M.R. (2007). An animal model for identifying a common stem/progenitor to liver cells and pancreatic cells. Patent: EP1857546; 2007a.

Sarvetnick, N., Krakowki, M.L. & Kritzik, M.R. Pancreatic progenitor cells and methods for isolating the same. Patent: US2007148706; 2007b.

Saudek, F., Havrdova, T., Boucek, P., Karasova, L,, Novota, P. & Skibova, J. (2004). Polyclonal anti-T-cell therapy for type 1 diabetes mellitus of recent onset. *Rev Diabet Stud*, Vol. 1, No. 2, (Summer 2004), pp. 80-88, ISSN 1614-0575

Schuh, A., Liehn, E.A., Sasse, A., Hristov, M., Sobota, R., Kelm, M., Merx, M.W. & Weber, C. (2008). Transplantation of endothelial progenitor cells improves neovascularization and left ventricular function after myocardial infarction in a rat model. *Basic Res Cardiol*, Vol. 103, No. 1, (January 2008), pp. 69-77, ISSN 0300-8428

Shapiro, A.M., Lakey, J.R., Ryan, E.A., Korbutt, G.S., Toth, E., Warnock, G.L., Kneteman, N.M. & Rajotte, R.V. (2000). Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med*, Vol. 343, No. 4, (July 2000), pp. 230-238, ISSN 0028-4793

Shapiro, A.M., Ricordi, C. & Hering, B. (2003). Edmonton's islet success has indeed been replicated elsewhere. *Lancet*, Vol. 362, No. 9391, (October 2003), pp. 1242, ISSN 0140-6736

Shapiro, A.M., Ricordi, C., Hering, B.J., Auchincloss, H., Lindblad, R., Robertson, R.P., Secchi, A., Brendel, M.D., Berney, T., Brennan, D.C., Cagliero, E., Alejandro, R., Ryan, E.A., DiMercurio, B., Morel, P., Polonsky, K.S., Reems, J.A., Bretzel, R.G., Bertuzzi, F., Froud, T., Kandaswamy, R., Sutherland, D.E., Eisenbarth, G., Segal, M., Preiksaitis, J., Korbutt, G.S., Barton, F.B., Viviano, L., Seyfert-Margolis, V., Bluestone, J. & Lakey, J.R. (2006). International trial of the Edmonton protocol for islet transplantation. *N Engl J Med*, Vol. 355, No. 13, (September 2006), pp. 1318-1330, ISSN 0028-4793

Shi, S. & Gronthos, S. (2003). Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J Bone Miner Res*, Vol. 18, No. 4, (April 2003), pp. 696-704, ISSN 0884-0431

Shibata, T., Naruse, K., Kamiya, H., Kozakae, M., Kondo, M., Yasuda, Y., Nakamura, N., Ota, K., Tosaki, T., Matsuki, T., Nakashima, E., Hamada, Y., Oiso, Y. & Nakamura, J. (2008). Transplantation of bone marrow-derived mesenchymal stem cells improves diabetic polyneuropathy in rats. *Diabetes*, Vol. 57, No. 11, (November 2008), pp. 3099-3107, ISSN 0012-1797

Shiroi, A., Yoshikawa, M., Yokota, H., Fukui, H., Ishizaka, S., Tatsumi, K. & Takahashi, Y. (2002). Identification of insulin-producing cells derived from embryonic stem cells by zinc-chelating dithizone. *Stem Cells*, Vol. 20, No. 4, pp. 284-292, ISSN 1066-5099

Smukler, S.R., Arntfield, M.E., Razavi, R., Bikopoulos, G., Karpowicz, P., Seaberg, R., Dai, F,, Lee, S., Ahrens, R., Fraser, P.E., Wheeler, M.B. & van der Kooy, D. (2011). The adult mouse and human pancreas contain rare multipotent stem cells that express insulin. Cell Stem Cell, Vol. 8, No. 3, (March 2011), pp. 281-93, ISSN 1934-5909

Staeva-Vieira, T., Peakman, M. & von Herrath, M. (2007). Translational mini-review series on type 1 diabetes: Immune-based therapeutic approaches for type 1 diabetes. *Clin Exp Immunol*, Vol. 148, No. 1, (April 2007), pp. 17-31, ISSN 0009-9104

Steiner L, Kröncke K, Fehsel K, & Kolb-Bachofen V. (1997). Endothelial cells as cytotoxic effector cells: cytokine-activated rat islet endothelial cells lyse syngeneic islet cells via nitric oxide. *Diabetologia*, Vol. 40, No. 2, (February 1997), pp. 150-155, ISSN 0012-186X

Suckale, J. & Solimena, M. (2008). Pancreas islets in metabolic signalling focus on the beta-cell. *Front Biosci*, Vol. 13, (May 2008), pp. 7156-7171, ISSN 1093-9946

Sun, T.T., Cotsarelis, G. & Lavker, R.M. (1991). Hair follicular stem cells: the bulge-activation hypothesis. *J Invest Dermatol*, Vol. 96, No. 5, (May 1991), pp. 77S-78S, ISSN 0022-202X

Suschek, C., Fehsel, K., Kröncke, K.D., Sommer, A. & Kolb-Bachofen, V. (1994). Primary cultures of rat islet capillary endothelial cells. Constitutive and cytokine-inducible macrophagelike nitric oxide synthases are expressed and activities regulated by glucose concentration. *Am J Pathol*, Vol. 145, No. 3, (September 1994), pp. 685-695, ISSN 0002-9440

Sutherland, D.E., Sibley, R., Xu, X.Z., Michael, A., Srikanta, A.M., Taub, F., Najarian, J. & Goetz, F.C. (1984). Twin-to-twin pancreas transplantation: reversal and reenactment of the pathogenesis of type I diabetes. *Trans Assoc Am Physicians*, Vol.97, pp. 80-87, ISSN 0066-9458

Sykes, M. (2009). Hematopoietic cell transplantation for tolerance induction: animal models to clinical trials. *Transplantation*, Vol. 87, No. 3, (February 2009), pp. 309-316, ISSN 0041-1337

Takahashi, K. & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*, Vol. 126, No. 4, (August 2006), pp. 663-676, ISSN 0092-8674

Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K. & Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*, Vol. 131, No. 5, (November 2007), pp. 861-872, ISSN 0092-8674

Tanaka, Y., Tsutsumi, A., Crowe, D.M., Tajima, S. & Morrison, W.A. (2000). Generation of an autologous tissue (matrix) flap by combining an arteriovenous shunt loop with artificial skin in rats: preliminary report. *Br J Plast Surg*, Vol. 53, No. 1, (January 2000), pp. 51-57, ISSN 0007-1226

Tateishi, K., He, J., Taranova, O., Liang, G., D'Alessio, A.C. & Zhang, Y. (2008). Generation of insulin-secreting islet-like clusters from human skin fibroblasts. *J Biol Chem*, Vol. 283, No. 46, (November 2008), pp. 31601-31607, ISSN 0021-9258

Temple S. (2001). The development of neural stem cells. *Nature*, Vol. 414, No. 6859, (November 2001), pp. 112-117, ISSN

Teramura, Y. & Iwata, H. (2010). Bioartificial pancreas microencapsulation and conformal coating of islet of Langerhans. *Adv Drug Deliv Rev*, Vol. 62, No. 7-8, (June 2010), pp. 827-840, ISSN 0021-9258

Till, J.E., McCulloch, E.A. & Siminovitch, L. (1964). A stochastic model of stem cell proliferation, based on the growth of spleen colony-forming cells. *Proc Natl Acad Sci U S A*, Vol. 51, (January 1964), pp. 29-36, ISSN 0027-8424

Smukler, S.R., Arntfield, M.E., Razavi, R., Bikopoulos, G., Karpowicz, P., Seaberg, R., Dai, F., Lee, S., Ahrens, R., Fraser, P.E., Wheeler, M.B. & van der Kooy, D. (2011). The adult mouse and human pancreas contain rare multipotent stem cells that express insulin. Cell Stem Cell vol. 8, No., 3 (Mar 2011), pp. 281-293, ISSN 1934-5909

Thorel, F., Népote, V., Avril, I., Kohno, K., Desgraz, R., Chera, S. & Herrera, P.L. (2010). Conversion of adult pancreatic alpha-cells to beta-cells after extreme beta-cell loss. *Nature*, Vol. 464, No. 7292, (April 2010), pp. 1149-1154, ISSN 0028-0836

Tropepe, V., Coles, B.L., Chiasson, B.J., Horsford, D.J., Elia, A.J., McInnes, R.R. & van der Kooy, D. (2000). Retinal stem cells in the adult mammalian eye. *Science*, Vol. 287, No. 5460, (March 2000), pp. 2032-2036, ISSN 0036-8075

Uibo, R. & Lernmark, A. (2008). GAD65 autoimmunity-clinical studies. *Adv Immunol*, Vol. 100, pp. 39-78, ISSN 0065-2776

Valdés-González, R.A., Dorantes, L.M., Garibay, G.N., Bracho-Blanchet, E., Mendez, A.J., Dávila-Pérez, R., Yechoor, V. & Chan, L. (2010). Minireview: beta-cell replacement therapy for diabetes in the 21st century: manipulation of cell fate by directed differentiation. *Mol Endocrinol*, Vol. 24, No. 8, (August 2010), pp. 1501, ISSN 0888-8809

van Laar, J.M. & Tyndall A. (2006). Adult stem cells in the treatment of autoimmune diseases. *Rheumatology (Oxford)*, Vol. 45, No. 10, (October 2006), pp. 1187-1193, ISSN 1462-0324

Wagner, R.T., Lewis, J., Cooney, A. & Chan, L. (2010). Stem cell approaches for the treatment of type 1 diabetes mellitus. *Transl Res*, Vol. 156, No. 3, (September 2010), pp. 169-179, ISSN 1931-5244

Wang, X., Zhao, T., Huang, W., Wang, T., Qian, J., Xu, M., Kranias, E.G., Wang, Y. & Fan, G.C. (2009). Hsp20-engineered mesenchymal stem cells are resistant to oxidative stress via enhanced activation of Akt and increased secretion of growth factors. *Stem Cells*, Vol. 27, No. 12, (December 2009), pp. 3021-3031, ISSN1066-5099

Weir, G.C. & Bonner-Weir, S. (2004). Five stages of evolving beta-cell dysfunction during progression to diabetes. *Diabetes*, Vol. 53 Suppl 3, (December 2004), S16-21, ISSN 0012-1797

Willcox, A., Richardson, S.J., Bone, A.J., Foulis, A.K. & Morgan, N.G. (2010). Evidence of increased islet cell proliferation in patients with recent-onset type 1 diabetes. *Diabetologia*, Vol. 53, No. 9, (September 2010), pp. 2020-2028, ISSN 0012-186X

Wu, Y., Chen, L., Scott, P.G. & Tredget, E.E. (2007). Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. *Stem Cells*, Vol. 25, No. 10, (October 2007), pp. 2648-2659, ISSN 1066-5099

Xu, X., D'Hoker, J., Stangé, G., Bonné, S., De Leu, N., Xiao, X., Van de Casteele, M., Mellitzer, G., Ling, Z., Pipeleers, D., Bouwens, L., Scharfmann, R., Gradwohl, G. & Heimberg, H. (2008). Beta cells can be generated from endogenous progenitors in injured adult mouse pancreas. *Cell*, Vol. 132, No. 2, (January 2008), pp.197-207, ISSN 0092-8674

Yang, L., Li, S., Hatch, H., Ahrens, K., Cornelius, J.G., Petersen, B.E., Peck, A.B. (2002). In vitro trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. *Proc Natl Acad Sci U S A*, Vol. 99, No. 12, (June 2002), pp. 8078-8083, ISSN 0027-8424

Yang, Z., Li, K., Yan, X., Dong, F. & Zhao, C. (2010). Amelioration of diabetic retinopathy by engrafted human adipose-derived mesenchymal stem cells in streptozotocin diabetic rats. *Graefes Arch Clin Exp Ophthalmol*, Vol. 248, No. 10, (October 2010), pp. 1415-1422, ISSN 0721-832X

Zappia, E., Casazza, S., Pedemonte, E., Benvenuto, F., Bonanni, I., Gerdoni, E., Giunti, D., Ceravolo, A., Cazzanti, F., Frassoni, F., Mancardi, G. & Uccelli, A. (2005). Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood*, Vol. 106, No. 5, (September 2005), pp. 1755-1761, ISSN 0006-4971

Zhang, N., Li, J., Luo, R., Jiang, J. & Wang, J.A. (2008). Bone marrow mesenchymal stem cells induce angiogenesis and attenuate the remodeling of diabetic cardiomyopathy. *Exp Clin Endocrinol Diabetes*, Vol. 116, No. 2, (February 2008), pp. 104-111, ISSN 0947-7349

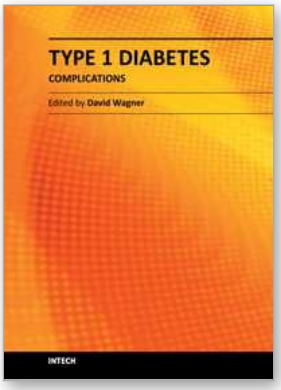
Zhao, M., Amiel, S.A., Ajami, S., Jiang, J., Rela, M., Heaton, N. & Huang, G.C. (2008). Amelioration of streptozotocin-induced diabetes in mice with cells derived from human marrow stromal cells. *PLoS One*, Vol. 3, No. 7, (July 2008), pp. e2666, ISSN 1932-6203

Zhao, Y., Lin, B., Darflinger, R., Zhang, Y., Holterman, M.J. & Skidgel, R.A. (2009). Human cord blood stem cell-modulated regulatory T lymphocytes reverse the autoimmune-caused type 1 diabetes in nonobese diabetic (NOD) mice. *PLoS One*, Vol. 4, No. 1, (January 2009), pp. e4226, ISSN 1932-6203

Zhao, Y. & Mazzone, T. (2010). Human cord blood stem cells and the journey to a cure for type 1 diabetes. *Autoimmun Rev*, Vol. 10, No. 2, (December 2010), pp. 103-107, ISSN 1568-9972

Zanone, M.M., Favaro, E. & Camussi, G. (2008). From endothelial to beta cells: insights into pancreatic islet microendothelium. *Curr Diabetes Rev*, Vol. 4, No. 1, (February 2008), pp. 1-9, ISSN 1573-3998

Zanone, M.M., Favaro, E., Miceli, I., Grassi, G., Camussi, E., Caorsi, C., Amoroso, A., Giovarelli, M., Perin, P.C. & Camussi, G. (2010). Human mesenchymal stem cells modulate cellular immune response to islet antigen glutamic acid decarboxylase in type 1 diabetes. *J Clin Endocrinol Metab*, Vol. 95, No. 8, (August 2010), pp. 3788-97, ISSN 0021-972X



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This book is a compilation of reviews about the complication of Type 1 Diabetes. T1D is a classic autoimmune disease. Genetic factors are clearly determinant but cannot explain the rapid, even overwhelming expanse of this disease. Understanding etiology and pathogenesis of this disease is essential. The complications associated with T1D cover a range of clinical obstacles. A number of experts in the field have covered a range of topics for consideration that are applicable to researcher and clinician alike. This book provides apt descriptions of cutting edge technologies and applications in the ever going search for treatments and cure for diabetes.

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