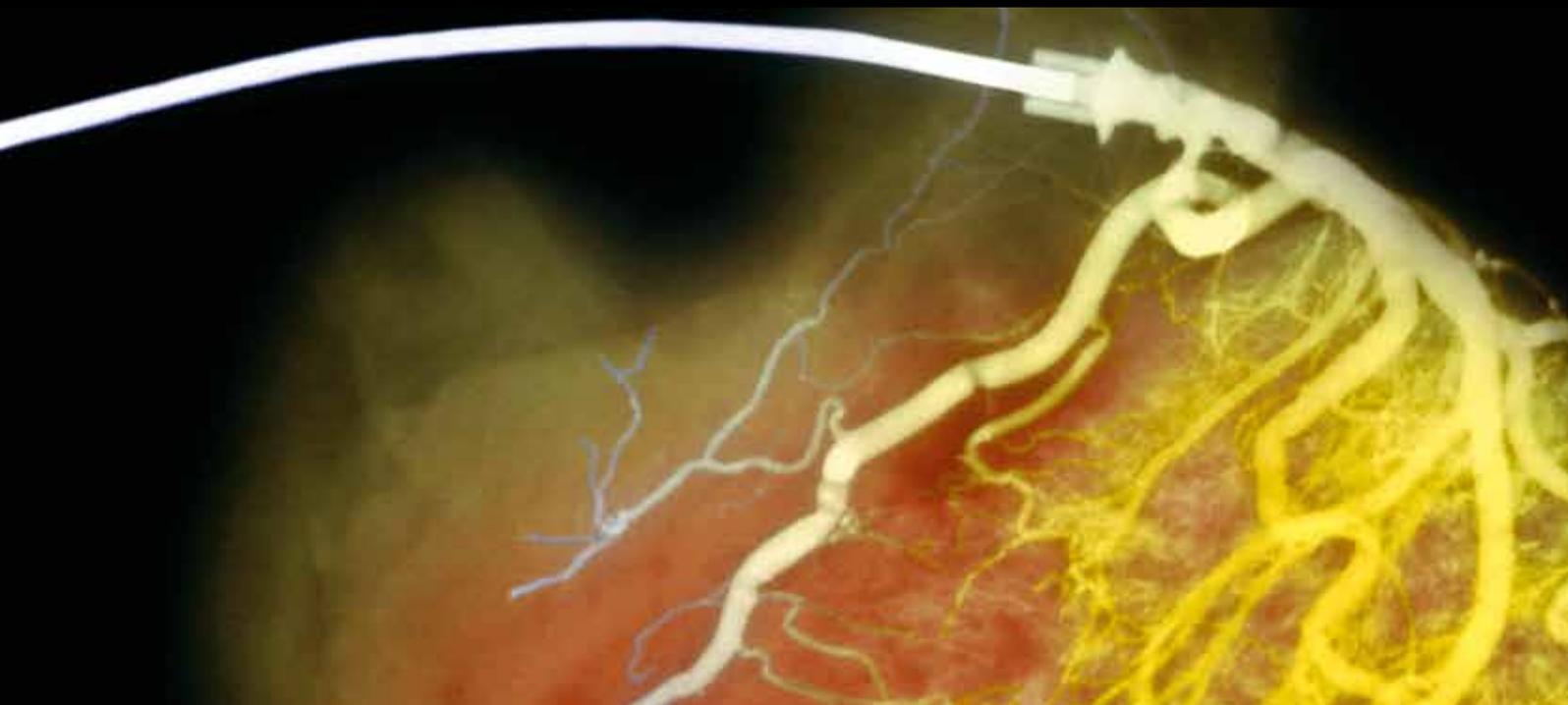


Islet Transplantation & β -Cell Replacement Therapies for Diabetes

GUEST EDITORS: ANTONELLO PILEGGI, THIERRY BERNEY, OLLE KORSGREN, AND ANDREW POSSELT





Islet Transplantation & β -Cell Replacement Therapies for Diabetes

Journal of Transplantation

Islet Transplantation & β -Cell Replacement Therapies for Diabetes

Guest Editors: Antonello Pileggi, Thierry Berney,
Olle Korsgren, and Andrew Posselt



Copyright © 2011 Hindawi Publishing Corporation. All rights reserved.

This is a special issue published in "Journal of Transplantation." All articles are open access articles distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Editorial Board

Gian Luigi Adani, Italy
Richard Allen, Australia
Joseph F. Buell, USA
P. Burra, Italy
Diego Cantarovich, France
Denis Castaing, France
Gaetano Ciancio, USA
F. H. J. Claas, The Netherlands
Waldo Concepcion, USA
J. V. Conte, USA
John H. Dark, UK
R. Davis, USA
Amrita Dosanjh, USA
R. Duquesnoy, USA

Niloo M. Edwards, USA
Yuri Genyk, USA
K. A. V. Hockerstedt, Finland
Ashokkumar Jain, USA
Bruce Kaplan, USA
R. L. Kormos, USA
Bernhard K. Krämer, Germany
Sheri M. Krams, USA
Paul C. Kuo, USA
Raymond H. Liang, Hong Kong
Malek G. Massad, USA
Shuji Miyagawa, Japan
P. S. Randhawa, USA
Camillo Ricordi, USA

Simon Robson, USA
Wojciech Rowiński, Poland
John Paul Scott, USA
Linda Sher, USA
Haval Shirwan, USA
A. Joseph Tector, USA
Eric Thervet, France
Giuseppe Tisone, Italy
G. Tufveson, Sweden
Raman V. Venkataramanan, USA
Trevor Williams, Australia
Kazuhiko Yamada, USA
Norio Yoshimura, Japan
Andreas Zuckermann, Austria

Contents

Islet Transplantation & β -Cell Replacement Therapies for Diabetes, Thierry Berney, Olle Korsgren, Andrew Posselt, and Antonello Pileggi
Volume 2011, Article ID 157840, 2 pages

Induction of Protective Genes Leads to Islet Survival and Function, Hongjun Wang, Christiane Ferran, Chiara Attanasio, Fulvio Calise, and Leo E. Otterbein
Volume 2011, Article ID 141898, 10 pages

Striated Muscle as Implantation Site for Transplanted Pancreatic Islets, Daniel Espes, Olof Eriksson, Joey Lau, and Per-Ola Carlsson
Volume 2011, Article ID 352043, 6 pages

Current Status of Immunomodulatory and Cellular Therapies in Preclinical and Clinical Islet Transplantation, Preeti Chhabra and Kenneth L. Brayman
Volume 2011, Article ID 637692, 24 pages

Evolution of β -Cell Replacement Therapy in Diabetes Mellitus: Islet Cell Transplantation, Cyrus Jahansouz, Cameron Jahansouz, Sean C. Kumer, and Kenneth L. Brayman
Volume 2011, Article ID 247959, 21 pages

Molecular Imaging: A Promising Tool to Monitor Islet Transplantation, Ping Wang, Zdravka Medarova, and Anna Moore
Volume 2011, Article ID 202915, 14 pages

B7-H4 Pathway in Islet Transplantation and β -Cell Replacement Therapies, Xiaojie Wang, Jianqiang Hao, Daniel L. Metzger, Ziliang Ao, Mark Meloche, C. Bruce Verchere, Lieping Chen, Dawei Ou, Alice Mui, and Garth L. Warnock
Volume 2011, Article ID 418902, 8 pages

β -Cell Generation: Can Rodent Studies Be Translated to Humans?, Françoise Carlotti, Arnaud Zaldumbide, Johanne H. Ellenbroek, H. Siebe Spijker, Rob C. Hoeben, and Eelco J. de Koning
Volume 2011, Article ID 892453, 15 pages

Engraftment of Insulin-Producing Cells from Porcine Islets in Non-Immune-Suppressed Rats or Nonhuman Primates Transplanted Previously with Embryonic Pig Pancreas, Marc R. Hammerman
Volume 2011, Article ID 261352, 7 pages

Pancreas Procurement and Preservation for Islet Transplantation: Personal Considerations, Hirofumi Noguchi
Volume 2011, Article ID 783168, 7 pages

Human Liver Cells Expressing Albumin and Mesenchymal Characteristics Give Rise to Insulin-Producing Cells, Irit Meivar-Levy, Tamar Sapir, Dana Berneman, Tal Weissbach, Sylvie Polak-Charcon, Philippe Ravassard, Andreas G. Tzakis, Eytan Mor, Camillo Ricordi, and Sarah Ferber
Volume 2011, Article ID 252387, 12 pages

Editorial

Islet Transplantation & β -Cell Replacement Therapies for Diabetes

Thierry Berney,¹ Olle Korsgren,² Andrew Posselt,³ and Antonello Pileggi⁴

¹ Department of Surgery, Geneva University Hospitals, 1211 Geneva, Switzerland

² Department of Clinical Immunology, Uppsala University Hospital, 75185 Uppsala, Sweden

³ Department of Surgery, University of California, San Francisco, CA 94143, USA

⁴ Departments of Surgery, Biomedical Engineering, Microbiology and Immunology, Diabetes Research Institute, University of Miami, Miami, FL 33136, USA

Correspondence should be addressed to Antonello Pileggi, apileggi@med.miami.edu

Received 23 January 2012; Accepted 23 January 2012

Copyright © 2011 Thierry Berney et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Restoration of physiologic metabolic control is the ultimate therapeutic goal for patients with diabetes but is difficult to achieve by means of medical therapy. Transplantation of islets of Langerhans represents a viable therapeutic option for patients with insulin-dependent diabetes. It is currently offered to patients with a brittle form of diabetes and hypoglycemia unawareness. Current limitations of islet transplantation include scarcity of deceased donor pancreata, partially effective islet isolation and purification protocols, need for life-long systemic immunosuppression, inadequate implantation sites, lack of predictive tests, and noninvasive graft monitoring protocols.

Great progress has been recorded in different research areas aimed at overcoming current limitations of islet cell transplantation. These areas include stem cells technology, xenotransplantation, development of novel immunosuppressive regimens, immune tolerance, immunoisolation protocols, alternative sites for islet implantation, immune monitoring, and noninvasive imaging techniques. This special issue was open to the scientific community working on the development of beta-cell replacement. Of the several papers received, ten received favorable peer reviews and were selected for publication in this issue to cover specific topics of general interest in this field.

The paper by C. Jahansouz et al. provides a comprehensive and historical overview of beta-cell replacement therapies. The paper by P. Chhabra and K. L. Brayman reviews emerging clinical and preclinical approaches toward enhancing the clinical outcome of islet cell transplantation

and includes aspects related to cytoprotection and immune interventions. The paper by H. Wang et al. is focused on important cytoprotective pathways such as carbon monoxide and heme oxygenase-1 and their ability to enhance islet cell viability and engraftment. The paper by X. Wang et al. offers a perspective on the B7-H4 co-stimulation pathway and its potential toward enhancing immune regulation and prolonging islet graft survival.

Recognizing the current limitations of human pancreas donation after brain death, alternative cellular-based approaches are actively being explored to widen the clinical application of beta-cell replacement therapies. The paper by M. R. Hammerman provides interesting new experimental data on porcine islet cell engraftment in xenogeneic recipients without immunosuppression. In his paper H. Noguchi shares an overview of his experience with novel protocols aimed at ameliorating pancreas preservation injury and improving the yield and quality of islets obtained for transplantation. I. Meivar-Levy et al. explore the use of hepatic cells expressing albumin and mesenchymal properties that have been engineered to produce insulin as an alternative and potentially unlimited source of transplantable insulin-producing cells. F. Carlotti et al. assess the important issue of the pros and cons related to the translatability of results obtained in rodent studies to humans when studying beta-cell regeneration.

There are increasing efforts aimed at developing novel implantation sites for islet cells. The paper by D. Espes

et al. describes the promising properties of intramuscular site which makes of it a viable option for islet cell implantation.

P. Wang et al. review the important and rapidly evolving field in vivo islet imaging. The use of molecular imaging techniques may be of invaluable assistance toward monitoring islet grafts as well as a tool to better understand the physiopathological changes that occur after transplantation.

*Thierry Berney
Olle Korsgren
Andrew Posselt
Antonello Pileggi*

Review Article

Induction of Protective Genes Leads to Islet Survival and Function

Hongjun Wang,¹ Christiane Ferran,² Chiara Attanasio,³ Fulvio Calise,³
and Leo E. Otterbein²

¹Division of General Surgery, Department of Surgery, Medical University of South Carolina, Charleston, SC 29425, USA

²Department of Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA

³Center of Biotechnologies, Cardarelli Hospital, 80131 Napoli, Italy

Correspondence should be addressed to Hongjun Wang, wanho@musc.edu

Received 10 June 2011; Accepted 1 September 2011

Academic Editor: Antonello Pileggi

Copyright © 2011 Hongjun Wang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Islet transplantation is the most valid approach to the treatment of type 1 diabetes. However, the function of transplanted islets is often compromised since a large number of β cells undergo apoptosis induced by stress and the immune rejection response elicited by the recipient after transplantation. Conventional treatment for islet transplantation is to administer immunosuppressive drugs to the recipient to suppress the immune rejection response mounted against transplanted islets. Induction of protective genes in the recipient (e.g., heme oxygenase-1 (HO-1), A20/tumor necrosis factor alpha inducible protein3 (tnfaip3), biliverdin reductase (BVR), Bcl2, and others) or administration of one or more of the products of HO-1 to the donor, the islets themselves, and/or the recipient offers an alternative or synergistic approach to improve islet graft survival and function. In this perspective, we summarize studies describing the protective effects of these genes on islet survival and function in rodent allogeneic and xenogeneic transplantation models and the prevention of onset of diabetes, with emphasis on HO-1, A20, and BVR. Such approaches are also appealing to islet autotransplantation in patients with chronic pancreatitis after total pancreatectomy, a procedure that currently only leads to 1/3 of transplanted patients being diabetes-free.

1. Introduction

Type 1 diabetes (T1D) is caused by the death of insulin-producing pancreatic β cells within the pancreas. Islet transplantation, a procedure that can restore the body's blood glucose level in a physiological manner, holds the most promise in treating patients with T1D [1]. With the success of the Edmonton protocol, clinical islet transplantation can provide T1D patients with sustained and improved glycemic control and a period of insulin independence [2]. There are, however, many problems with this procedure. First, nonimmune-related stress during islet isolation and transplantation results in a significant number of islets undergoing apoptosis immediately after transplantation. Thus, at least 2-3 donors are needed per recipient to ensure survival of a sufficient islet cell mass to achieve insulin independence [3-6]. Second, those islets that survive need to sustain an allograft rejection response and recurrence of autoimmunity mediated by the recipients' T cells, natural killer cells, monocytes, and

cytokines, otherwise additional islet/ β cell death would ensue [7]. Both obstacles have significantly limited clinical application of islet transplantation for the treatment of T1D. Similarly, the effectiveness of autologous islet transplantation, a procedure currently implemented in the clinic to treat patients suffering from chronic pancreatitis, is also impacted by β cell apoptosis posttransplantation, that is, only 1/3 of the patients are insulin-free after total pancreatectomy and islet autotransplantation [8-10]. Donor islet quality plays a critical role in determining the outcome of allo- and autotransplantation of islet grafts, with stress-induced β cell apoptosis greatly contributing to failure of these procedures. Thus, novel strategies that enable β cell resistance to stress would prevent β cell apoptosis and reduce or even eliminate immune rejection and recurrent autoimmunity thereby benefiting clinical application of islet transplantation.

The etiology of T1D is complex and poorly understood. Many factors including genetic susceptibility, environmental factors, the immune system, and β cells themselves were

found to participate in the pathogenic process of this disorder [11]. A variety of pathogenic pathways including CD8⁺ cytolytic T-cell-mediated killing, cytokine exposure, apoptosis caused by fatty acid synthase and fatty acid synthase ligand can lead to immune-mediated destruction of β cells during the onset of T1D [12], suggesting that individual therapeutic strategies targeting one pathway may not be sufficient to cure T1D [13, 14].

A protective gene is a gene that is upregulated in response to stress through specific signaling cascades and transcription factor regulation that when induced participate in promoting cell survival [15] (Figure 1). Many protective genes including HO-1, A20, B-cell lymphoma 2 (Bcl-2), Bcl-x, heat shock proteins, biliverdin reductase (BVR), and antioxidant enzymes have been found to be expressed in pancreatic islets, and their expression leads to protection against apoptosis and other injuries while their absence leads to a heightened response to stress or in the case of HO-1, low fecundity, and a shortened lifespan fraught with continuous inflammatory sequelae throughout life [16], and in the case of A20 unfettered inflammation and death within 3–6 weeks of birth [17].

2. HO-1

HO-1 is the rate-limiting enzyme that degrades heme to generate equal molar amounts of carbon monoxide (CO), biliverdin, and iron [18]. Biliverdin is rapidly converted into bilirubin by biliverdin reductase, and iron is sequestered into ferritin. HO-1 is a ubiquitous stress protein and can be induced in many cell types by various stimuli [19]. There is increasing evidence indicating that induction of HO-1 provides cellular protection against transplant rejection [20, 21], hypertension [22], hyperoxia [23], acute pleurisy [24], ischemia reperfusion injury [25], and endotoxic shock [22]. HO-1 is intimately involved in the inflammatory, apoptotic, and proliferative properties of the cell in response to a given stress. The anti-inflammatory properties ascribed to HO-1 are an important means of protection and survival. Mice deficient in HO-1 develop a chronic inflammatory state that progresses with age. The first HO-1 deficient human died of an inflammatory syndrome at the age of six [26, 27]. Another case of human HO-1 deficiency was reported recently in a young girl with congenital asplenia, who presented with multiple organ dysfunction as well as hemolysis, inflammation, nephritis, and resistance to therapy [28]. There is evidence that each product of HO-1, biliverdin/bilirubin, CO, or ferritin accounts for its protective effects both when used alone or in combination [29–32].

3. HO-1 Increases Survival and Function of Islet Allograft

More than half of the islet tissue is lost in both the syngeneic and the autoimmune transplantation settings at 2–3 days posttransplantation, which contributed to the primary nonfunction of transplanted islets [33]. Considering the shortage of islet donors, prevention of β cell apoptosis will effectively reduce the number of donors required for each transplant and increase the success rate for this procedure.

Early islet/ β cell apoptosis after transplantation is typically induced by nonimmune-mediated stressors including prolonged hypoxia during the revascularization process, nutrition deprivation, ischemia reperfusion injuries, and proinflammatory and cytokine expression [5]. HO-1 expression is observed in islets under stress conditions, such as during islet isolation prior to transplantation or cytokine treatment with IL-1 β and IFN γ [6]. Induction of HO-1 pharmacologically or via gene transfer protects islets from stress-induced apoptosis in both the *in vitro* and the *in vivo* settings. *In vitro*, several studies have showed that HO-1 induction in β cell lines, primary murine, or human islets protects against apoptosis induced by TNF- α and cyclohexamide (CHX), interleukin-1 β (IL-1 β), and Fas [34–36]. Transduction of HO-1 with a TAT protein transduction domain (TAT/PTD), an 11-aa cell penetrating peptide from the human immunodeficiency virus TAT protein, into islets, improves islet viability in culture. HO-1 has been also shown to prevent β -cell apoptosis via p38 MAPK activation and the NF- κ B pathway in this study [37].

In addition to the *in vitro* experiments described above, Pileggi et al. showed that induction of HO-1 pharmacologically with cobalt protoporphyrin (CoPP) in recipients results in improved islet function in a marginal mass islet transplantation model in rodents, that is, fewer islets are required to achieve normoglycemia when transplanted into a syngeneic recipient that have been rendered diabetic by streptozotocin (STZ) treatment [36]. In addition, a short course of CoPP administration to recipients leads to long-term survival of DBA/2 (H-2^d) islets in 30% of diabetic C57BL/6 (H-2^b) recipients [38]. Most importantly, tolerance to transplanted islets is achieved as long-term graft-bearing animals rejected third-party islets while accepting a second-set donor-specific graft permanently, without additional treatment. It seems that induction of HO-1 leads to a donor-specific hyporesponsiveness in the CoPP-treated animals. Additionally, there is greatly reduced class II expression and a transient and powerful immunosuppression observed with reduced lymphocyte proliferative responses and increased proportions of T regulatory cells with decreased mononuclear cell infiltration into the graft [38, 39].

Another critical finding in the Pileggi study is that preconditioning of islets with hemin to induce HO-1 activity leads to improved graft survival in untreated recipients. Moreover, islet preconditioning provides additional advantages in HO-1-induced recipients that results in an increased proportion of long-term survival of transplanted islet allografts in recipients. Encouraged by this study, we tested whether HO-1 induction, or CO administration, to the islet donor, would sustain survival and function of transplanted islet allografts. Such an approach would avoid the toxicity associated with recipient treatment. Our data showed that without any other treatment, induction of HO-1 (20 mg/kg CoPP, 24 hr before isolation) or administering CO (250 ppm for 1 hr) only to the donor leads to long-term survival of DBA/2 (H-2^d) islets in diabetic B6AF1 (H-2^{b,k/d}) recipients, which are then antigen specifically tolerant. In essence, by using CO, we were mimicking the effects of HO-1 itself with one of its products. Several

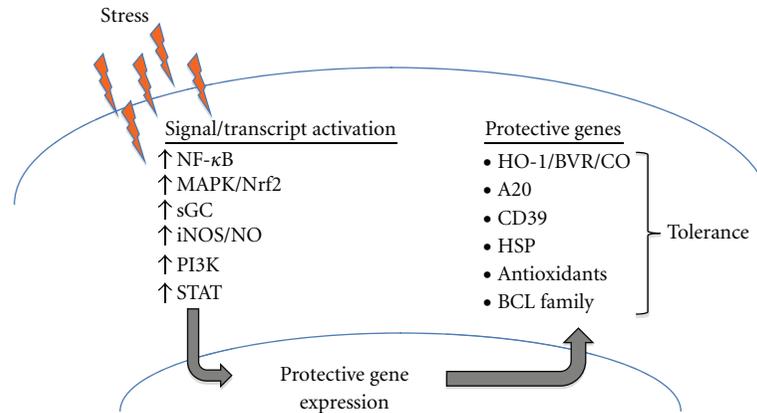


FIGURE 1

proinflammatory and proapoptotic genes that are strongly induced in islets after transplantation in the untreated situation were significantly suppressed after administering CO to the donor. These included TNF- α , inducible nitric oxide synthase (iNOS), monocyte chemoattractant protein-1 (MCP-1), granzyme B, and Fas/Fas ligand, all of which contribute to the pathogenesis and rejection of transplanted islets. Moreover, donor treatment is correlated with less infiltration of recipient macrophages into the transplanted islets [40]. We tested further whether CO conferred protection by suppressing Toll-like receptor 4 (TLR4) upregulation in pancreatic β cells. TLR4 is normally activated in islets during the isolation procedure, and its activation allows initiation of inflammation, which leads to islet allograft rejection. Donor treatment with CO suppresses TLR4 expression in freshly isolated islets as well as in transplanted islets at various times after transplantation. Islet allografts from TLR4-deficient mice survive indefinitely in BALB/c recipients and show significantly less inflammation after transplantation compared with grafts from a control donor. Isolated islets preinfected with a TLR4 dominant negative mutant virus before transplantation demonstrated prolonged survival in recipients. Despite the salutary effects of TLR4 suppression, HO-1 expression is still needed in the recipient for islet survival: TLR4-deficient islets were rejected promptly after being transplanted into recipients in which HO-1 activity was blocked [41]. Our data suggest that TLR4 induction in β cells is involved in β cell death and graft rejection after transplantation. CO exposure protects islets from rejection in part by blocking TLR4 upregulation in β cells.

There are at several mechanisms by which HO-1 functions in the islet allogeneic transplantation model. First, HO-1 induction leads to a decreased inflammatory response in transplanted islets as compared to islets harvested from untreated donors. Inflammation not only contributes to β apoptosis but also heightens the alloaggressive immune response; thus, suppression of inflammation can lead to fewer cell deaths and a lesser immune rejection response. Second, the diminution of free radicals by HO-1 and its products should impart salutary effects as islet cells express lower levels of antioxidant genes than most other tissues of the body and are extremely sensitive to oxidative damages

[42]. Third, HO-1 induction in the recipient increases the number/function of T regulatory cells, which generate a favorable microenvironment to transplanted islets and eventually contribute to the survival of those islets. Last but not least, HO-1 induction leads to the generation of biliverdin/bilirubin, requisite activation of BVR, and generation of CO, which can amplify the protective effects of HO-1 as CO is anti-inflammatory and antiapoptotic and the bile pigments are strong antioxidants and BVR can function to quell the inflammatory response [42]. Emerging data clearly demonstrate that BVR can regulate the inflammatory response through distinct intracellular signaling activity leading to increases in the anti-inflammatory cytokine IL-10 [43].

4. Overexpression of HO-1 Improves Function of an Islet Xenograft

The success of islet allogeneic transplantation is limited by the number of organ donors. Xenogenic donors (e.g., pig) offer potential unlimited sources of islets, and islet xenotransplantation is an alternative option for patients with T1D. However, despite a number of widely recognized advantages, the clinical application of porcine islet xenotransplantation has been hindered by a potent recipient xenospecific immune response and by the lack of a tolerable immunosuppressive strategy to overcome this barrier since cellular immune responses to xenogeneic cells are less clear and poorly understood [44–46]. A key for immunologic rejection in xenotransplantation is the damage to the graft due to chemotactic movement and infiltration of leukocytes into the graft [47]. The protective effects of HO-1 in the islet xenotransplantation model have been investigated by several groups, and improved survival and function of islet xenograft was observed when HO-1 was induced. For example, in a rat to mouse islet transplantation model, incubation of rat islets with CoPP before transplantation leads to a much better glucose-induced insulin secretion, longer graft survival time (14.63 ± 1.19 day versus 9.88 ± 2.17 days in control group), and less lymphocyte infiltration into the graft [48]. These results were confirmed by another group in which HO-1 was induced in male Sprague Dawley

donor rats before islet isolation and transplanting the islets into C57BL/6 mice rendered diabetic by streptozotocin. Again, improved graft survival was observed [49]. In both studies, less lymphocyte infiltration and elevated IL-10 expression were observed in HO-1-induced islet xenografts, a phenomenon also observed in other models of HO-1 action (e.g., HO-1 increases expression of IL-10). IL-10 functions as a negative immunomodulatory factor and participates actively during inflammation, tumor immune responses, and the transplantation immune response. It promotes activation and differentiation of B cells, mediates humoral immunity, and inhibits proinflammatory cytokine expression and mononuclear cell expression of MHC II molecules and costimulatory molecules, as well as cytokine synthesis [50, 51]. Many of the observed protective effects of HO-1 in the xenotransplantation model might be mediated by IL-10 as HO-1 generates CO, which downregulated iNOS and upregulates IL-10 [15] and leads to protection to β cells.

5. HO-1 Induction Interferes with the Onset of Diabetes

The pathophysiology of T1D is characterized by dysfunction and death of insulin-producing β cells in the pancreatic islets of Langerhans. At an early stage of disease onset, progressive mononuclear cells invade the islets and cause insulinitis, a process that lasts for several weeks to months before severe β cell destruction occurs [52]. Mononuclear cell infiltration leads to the generation of reactive oxygen species (ROS) and proinflammatory cytokines including IL-1 β , INF- γ , and TNF- α in pancreatic β cells. Elevated intracellular levels of ROS, including superoxide, hydrogen peroxide, and nitric oxide, leads to apoptotic and necrosis of β cells [53]. Increased proinflammatory cytokines contribute to insulinitis [54]. The autoimmune nonobese diabetic (NOD) mouse resulting from autoreactive T-cell-mediated destruction of β cells is a useful and powerful model by which to study the development of T1D due to its similarity to human disease. Similar to human diabetes, the NOD mice develop insulinitis that has been linked to activated macrophages and T cells the secretion of soluble mediators, such as oxygen radicals, NO, and cytokines [55]. HO-1 has been shown to slow progression to overt diabetes and interdict disease progress. Li and colleagues reported that HO-1 induction in NOD mice by weekly injection of CoPP reduces hyperglycemia and preserves the number of β cells via suppressing infiltration of CD11c⁺ cells. Increased phosphorylation of AKT, Bcl-XL, and RSK levels and decreases in superoxide and 3-NT levels were observed in mice where HO-1 was induced [56]. The effect of HO-1 in preventing progression of overt diabetes was confirmed by another study in which HO-1 was induced in female NOD mice at 9 weeks of age with a single intravenous injection of a recombinant adeno-associated virus bearing the HO-1 gene (AAV-HO-1, 0.5×10^{10} – 2.5×10^{10} viruses/mouse). HO-1 induction significantly reduced destructive insulinitis and the incidence of overt diabetes examined over a 15-week period. HO-1-mediated protection was associated with a lower type 1 T-helper-cell-mediated

response. Adaptive transfer experiments into NOD-scid mice demonstrated that splenocytes isolated from AAV-HO-1 treated mice were less diabetogenic. However, no differences in CD4⁺CD25⁺ T regulatory cell infiltrates between saline-treated and the AAV-HO-1 treated group was observed [57]. In both studies discussed above, the protective effects of HO-1 could be substituted for with bilirubin and/or CO.

Huang et al. confirmed the protective effect of HO-1 in preventing the onset of diabetes by generation of transgenic NOD mice in which the HO-1 transgene was driven by an insulin promoter (Plns-mHO-1) [58]. Although the overall expression level of HO-1 in transgenic islets was lower than that in nontransgenic islets stimulated with CoPP, a dramatic difference in insulinitis and a lower incidence of diabetes were observed in the transgenic mice. Onset of diabetes was significantly delayed in the mHO-1-transgenic NOD mice, that is, spontaneous diabetes developed after 15 weeks of age in the mHO-1-transgenic NOD mouse compared to 12 weeks in age-matched controls. Diabetic incidence at 30 weeks of age was also significantly reduced (33.3% in the transgenic mice compared to 66.7% in controls). Moreover, islets from transgenic mice survived significantly longer than those harvested from wild-type donors when transplanted into new onset spontaneous diabetic female NOD recipients, although permanent protection from recurrence of diabetes was not achieved in this model [58]. The mHO-1-transgenic NOD islet grafts still expressed HO-1 at day 8 after transplantation. Preservation of islet architecture and intact insulin-secreting islets were observed within the pancreas [58]. However, local expression of HO-1 did not alter systemic or local lymphocyte and dendritic cell development in NOD mice which was in contrast to studies by another group where HO-1 was shown to inhibit maturation of dendritic cells and regulate the function of Th1 and Treg cells [59]. Conclusions from these studies were that the anti-inflammatory and antioxidant properties of HO-1 and its products interfered with the onset of diabetes in NOD mice.

6. HO-1 Induction Increases Insulin Sensitivity

The islets of Langerhans are equipped with a HO-CO pathway which constitutes a regulatory system of physiologic importance for the stimulation of insulin and glucagon release [60]. HO-1 expression and activity are reduced in patients with T2D compared to healthy individuals [61]. Overexpression of HO-1 activates the insulin-signaling pathway and has been shown to have unique and long-lasting antidiabetic effects in the rodent model of insulin resistance [62–64]. Moreover, HO-1 attenuates the oxidative destruction of adiponectin/insulin and improves insulin sensitivity and glucose metabolism in the STZ-induced T1D mouse model [65]. Induction of HO-1 by hemin increases plasma insulin level and enhances insulin sensitivity and improves glucose tolerance. The antidiabetic effects of hemin lasted for 2 months after termination of therapy and were accompanied by enhanced HO-1 expression and HO-1 activity of the soleus muscle, along with potentiation of plasma antioxidants including bilirubin, ferritin, and superoxide dismutase with elevation of the total antioxidant

capacity. Hemin blocked C-Jun NH2-terminal kinase (JNK), a substance known to inhibit insulin biosynthesis, and suppressed markers/mediators of oxidative stress including 8-isoprostane, NF- κ B, and activating protein (AP-1 and AP-2) in the soleus muscle. In addition, hemin therapy significantly attenuated pancreatic histopathological lesions including acinar cell necrosis, interstitial edema, vacuolization, fibrosis, and mononuclear cell infiltration [66]. Thus, it seems that hemin-induced HO-1 can enhance the function of β cells via increase insulin sensitivity in the insulin resistance mouse model.

HO-1 and its products are also protective against diabetes-related complications. Human HO-1 cDNA transferred into diabetic rats restored mitochondrial ADP/ATP and deoxynucleotide carriers [67]. Elevated HO-1 was associated with a significant increase in the phosphorylation of AKT and levels of Bcl-XL proteins. The cytoprotective mechanisms of HO-1 against oxidative stress involve an increase in the number of macrophages and antiapoptotic proteins as well as cytochrome c oxidase activity in this model [67]. Moreover, exogenous administration of the CO releasing molecule-3 (CORM)-3 and bilirubin prevents endothelial cell sloughing in diabetic rats, likely via a decrease in oxidative stress which represents a novel approach to prophylactic vascular protection in diabetics [64, 67, 68]. In addition to functioning as a positive modulator of glucose-stimulated insulin release, CO increases the propagation of Ca²⁺ signals with coordinating effects on the β cell rhythmicity [69].

7. A20 and Islet Survival and Function

A20, also known as the TNF- α -induced protein 3 (TNFAIP3), is a zinc-ring finger protein that was first identified as a cytokine-induced gene in human umbilical vein endothelial cells [70]. As a negative regulator of nuclear factor kappa B (NF- κ B) activation, A20 is recognized as a central and ubiquitous regulator of inflammation and as a potent antiapoptotic gene in certain cell types, including β cells [71–73]. A20 offers a potential therapeutic target for the treatment of diseases where apoptosis and/or the inflammatory response constitute components of the pathophysiology; thus, it is an ideal cytoprotective gene therapy candidate for T1D [74]. Overexpression of A20 by means of adenovirus-mediated gene transfer protects islets from IL-1 β /INF- γ and Fas-induced apoptosis [75–77]. Transplantation of a suboptimal number of islets overexpressing A20 resulted in a cure in a high percentage of recipients compared to control islets. A20-expressing islets preserved functional β cell mass and are protected from cell death. The cytoprotective effect of A20 against apoptosis correlates with and is dependent on the abrogation of cytokine-induced NO production due to transcriptional blockade of iNOS induction; these data demonstrate a dual antiapoptotic and antiinflammatory function for A20 in β cells.

8. Biliverdin Reductase and Islet Protection

The breakdown of heme continues with biliverdin as it is rapidly converted to bilirubin by BVR. BVR has, in

recent years, evolved into a complex enzyme with additional functions including signal transduction and transcription factor activity. We include it here as many of the effects of HO-1 might be attributed in part to the additional functions for BVR resulting from the presence of biliverdin. A direct link between BVR and HO-1 in oxidative stress was described by Miralem et al. showing an attenuated HO-1 response to superoxide anion and arsenite in cells where BVR expression had been silenced [78]. BVR is a unique enzyme because it has been categorized to possess numerous biological functions. The reductase activity leads to the protective effects shown by biliverdin/bilirubin in a variety of experimental models of organ transplantation, endotoxic shock, and vascular injury [24]. BVR also exhibits kinase activity and corresponding signal transduction and more recently nuclear targeting and ability to regulate gene expression and the inflammatory response. Indeed, recent studies by us show that BVR is present on the cell surface and in this location binds biliverdin and in the conversion to bilirubin, activates a signal cascade leading to activation of Akt that in turn increases IL-10 expression [43]. The rapid conversion of biliverdin into bilirubin by BVR likely explains the beneficial effects observed with exogenous biliverdin administration. Indeed the signalling and transcriptional activity of BVR in addition to generating increased bilirubin may act synergistically and explain the mechanism of biliverdin-induced protection. Modulation of BVR itself directly regulates the inflammatory response and, *in vivo*, can prevent acute liver injury [43].

In addition to its anti-inflammatory effects, BVR modulates glucose uptake and insulin resistance by decreasing glucose transport and metabolism in competition with the insulin receptor substrate-1 (IRS-1) for phosphorylation by insulin receptor kinase (IRK). This leads to a reduced binding with PI3 K and accelerated degradation of IRS [79]. Therefore, therapeutic molecules designed to suppress the kinase activity of BVR may play an important role in the reversal of diabetes.

As previously discussed, islet allografts suffer a gradual loss of function in response to oxidative stress, inflammation, and apoptosis as well as activation of the humoral immune response. Although intraportal infusion represents the most frequent procedure in the clinic for human islet transplant, a high percentage of islets are destroyed at a very early posttransplant stage because of the instant blood-mediated inflammatory response [80, 81]. Bilirubin administration reduced apoptosis and improved insulin secretion in an *in vitro* model in INS-1 cells when challenged with nonspecific inflammation induced by cytokines. Moreover, bilirubin administration led to improved glucose control and protection of islets grafts in a syngeneic rat model of intraportal islet transplantation by inhibiting the production of IL-1 β , TNF- α , ICAM-1, and MCP-1, as well as infiltration of Kupffer cells [82].

Bilirubin administration to the donor, and even more so to cultured islets, without further treatment of the recipient would represent a great advantage in clinical practice. Freshly isolated islets from bilirubin-treated donors led to a strong expression of the protective genes HO-1 and bcl-2 and a clear suppression of the proapoptotic and proinflammatory

genes caspase-3, caspase-8, and MCP-1 [83, 84]. This protective effect of bilirubin leads to reduced β -cell destruction after transplantation, reduced macrophages infiltration, and decreased expression of MCP-1, BID, caspase-3, -8, and -9, TNF- α , iNOS, Fas, TRAIL-R, and CXCL10 in the graft after allogeneic transplantation [84]. The therapeutic potential of bilirubin is further corroborated by data reported in Gunn rats (genetically predisposed to high bilirubin levels) rendered diabetic by streptozotocin administration in which the typical hyperbilirubinemia represents a “natural” protection to oxidative stress [85].

Bilirubin administration to recipients clearly improves graft survival by inducing immune tolerance via *de novo* generation of T regulatory cells. Bilirubin was no longer protective when CD4⁺CD25⁺ Treg cells were depleted from recipients prior to transplantation suggesting that Tregs were critical in the ability of bilirubin to protect [86]. Moreover, as previously shown in kidney and heart transplantation models, dual therapy by combining CO and biliverdin enhanced long-term graft survival [87]. Interestingly, a recent study in a rodent model of type 2 diabetes describes the protective effects of biliverdin administered orally [88]. Biliverdin inhibited β -cell injury caused by oxidative stress and resulted in glucose tolerance and improved function. Biliverdin has been shown to increase the insulin content, reduce Bax, and enhance Pdx1 expression in diabetic mice compared to control [88]. Similar effects in T1D models which would be a significant turning point for potential clinical use have not yet been tested.

9. Other Protective Genes/Factors that Can Increase Islet Survival and Function

There are many other protective genes that have been shown to protect pancreatic β cells. Mancarella et al. reported that exposing human islets to the nonpeptidyl low molecular weight radical scavenger IAC [bis(1-hydroxy-2,2,6,6-tetramethyl-4-piperidyl) decanedioate dihydrochloride] on isolated human islet cells protected them from isolation and culture-induced oxidative stress [89]. Enhancing expression of suppressor of cytokine signaling 1 (SOCS1) in isolated rat islets prior to transplantation protected them from apoptotic loss and prolonged survival [90]. Transduction of NOD islets with the antioxidative gene thioredoxin (TRX, reactive oxygen species scavenger and antiapoptotic) using a lentiviral vector before transplantation prolonged islet graft survival in NOD mice [91]. Anthocyanins from Chinese Bayberry protects β cells against hydrogen-peroxide-induced necrosis and apoptosis via upregulation of HO-1 [92]. Adenoviral transfection of human islets with human X-linked inhibitor of apoptosis provided protection from inflammatory cytokines and improved their viability and function [2, 93–104].

10. Conclusion

Due to the complex nature of the pathogenesis of diabetes, interfering with antigenic recognition and/or cell death, imparting tolerance, immunoregulation, and cell protection offer a promising form of immunotherapy [3, 105]. Based on

the potent cytoprotective and immunoregulatory effects of HO-1, A20, BVR, and other protective genes, targeting strategies aimed to induce their expression or by administering one or more of their products hold great promise in protecting islet cells from apoptosis and may prove critical as potential therapies for diabetes and other human diseases.

Abbreviations

T1D:	Type 1 diabetes
HO-1:	Heme oxygenase-1
BVR:	Biliverdin reductase
IL-1 β :	Interleukin-1 β
CoPP:	Cobalt protoporphyrin
NOD:	Nonobese diabetic
STZ:	Streptozotocin
CORM:	CO releasing molecule
TLR4:	Toll-like receptor 4
iNOS:	Inducible nitric oxide synthase
MCP-1:	Monocyte chemoattractant protein-1.

References

- [1] S. S. M. S. Monfared, B. Larijani, and M. Abdollahi, “Islet transplantation and antioxidant management: a comprehensive review,” *World Journal of Gastroenterology*, vol. 15, no. 10, pp. 1153–1161, 2009.
- [2] J. A. Emamaullee and A. M. J. Shapiro, “Interventional strategies to prevent β -cell apoptosis in islet transplantation,” *Diabetes*, vol. 55, no. 7, pp. 1907–1914, 2006.
- [3] M. H. Kapturczak, T. Flotte, and M. A. Atkinson, “Adeno-Associated Virus (AAV) as a vehicle for therapeutic gene delivery: improvements in vector design and viral production enhance potential to prolong graft survival in pancreatic islet cell transplantation for the reversal of type 1 diabetes,” *Current Molecular Medicine*, vol. 1, no. 2, pp. 245–258, 2001.
- [4] M. Biarnés, M. Montolio, V. Nacher, M. Raurell, J. Soler, and E. Montanya, “ β -cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia,” *Diabetes*, vol. 51, no. 1, pp. 66–72, 2002.
- [5] A. M. Davalli, Y. Ogawa, C. Ricordi, D. W. Scharp, S. Bonner-Weir, and G. C. Weir, “A selective decrease in the beta cell mass of human islets transplanted into diabetic nude mice,” *Transplantation*, vol. 59, no. 6, pp. 817–820, 1995.
- [6] Y. B. Ahn, G. Xu, L. Marselli et al., “Changes in gene expression in beta cells after islet isolation and transplantation using laser-capture microdissection,” *Diabetologia*, vol. 50, no. 2, pp. 334–342, 2007.
- [7] E. Tobiasch, L. Günther, and F. H. Bach, “Heme oxygenase-1 protects pancreatic β cells from apoptosis caused by various stimuli,” *Journal of Investigative Medicine*, vol. 49, no. 6, pp. 566–571, 2001.
- [8] T. Kobayashi, J. C. Manivel, A. M. Carlson et al., “Correlation of histopathology, islet yield, and islet graft function after islet autotransplantation in chronic pancreatitis,” *Pancreas*, vol. 40, pp. 193–199, 2011.
- [9] J. Dixon, M. Delegege, K. A. Morgan, and D. B. Adams, “Impact of total pancreatectomy with islet cell transplant on chronic pancreatitis management at a disease-based center,” *American Surgeon*, vol. 74, no. 8, pp. 735–738, 2008.
- [10] D. S. Nath, T. A. Kellogg, and D. E. R. Sutherland, “Total pancreatectomy with intraportal auto-islet transplantation

- using a temporarily exteriorized omental vein,” *Journal of the American College of Surgeons*, vol. 199, no. 6, pp. 994–995, 2004.
- [11] J. A. Bluestone, K. Herold, and G. Eisenbarth, “Genetics, pathogenesis and clinical interventions in type 1 diabetes,” *Nature*, vol. 464, no. 7293, pp. 1293–1300, 2010.
 - [12] H. E. Thomas, M. D. McKenzie, E. Angstetra, P. D. Campbell, and T. W. Kay, “Beta cell apoptosis in diabetes,” *Apoptosis*, vol. 14, no. 12, pp. 1389–1404, 2009.
 - [13] D. Schatz, E. A. M. Gale, and M. A. Atkinson, “Why can’t we prevent type 1 diabetes?: maybe it’s time to try a different combination,” *Diabetes Care*, vol. 26, no. 12, pp. 3326–3328, 2003.
 - [14] B. O. Roep and M. Peakman, “Surrogate end points in the design of immunotherapy trials: emerging lessons from type 1 diabetes,” *Nature Reviews Immunology*, vol. 10, no. 2, pp. 145–152, 2010.
 - [15] L. E. Otterbein, M. P. Soares, K. Yamashita, and F. H. Bach, “Heme oxygenase-1: unleashing the protective properties of heme,” *Trends in Immunology*, vol. 24, no. 8, pp. 449–455, 2003.
 - [16] D. Ross Laybutt, H. Kaneto, W. Hasenkamp et al., “Increased expression of antioxidant and antiapoptotic genes in islets that may contribute to β -cell survival during chronic hyperglycemia,” *Diabetes*, vol. 51, no. 2, pp. 413–423, 2002.
 - [17] E. G. Lee, D. L. Boone, S. Chai et al., “Failure to regulate TNF-induced NF- κ B and cell death responses in A20-deficient mice,” *Science*, vol. 289, no. 5488, pp. 2350–2354, 2000.
 - [18] M. D. Maines, G. M. Trakshel, and R. K. Kutty, “Characterization of two constitutive forms of rat liver microsomal heme oxygenase. Only one molecular species of the enzyme is inducible,” *Journal of Biological Chemistry*, vol. 261, no. 1, pp. 411–419, 1986.
 - [19] R. Gozzelino, V. Jeney, and M. P. Soares, “Mechanisms of cell protection by heme oxygenase-1,” *Annual Review of Pharmacology and Toxicology*, vol. 50, pp. 323–354, 2010.
 - [20] R. Öllinger and J. Pratschke, “Role of heme oxygenase-1 in transplantation,” *Transplant International*, vol. 23, no. 11, pp. 1071–1081, 2010.
 - [21] K. Jiang, L. Cheng, J. Wang, J. Li, and J. Nie, “Heme oxygenase-1 expression in rats with acute lung rejection and implication,” *Journal of Huazhong University of Science and Technology—Medical Science*, vol. 29, no. 1, pp. 84–87, 2009.
 - [22] N. Hill-Kapturczak, S. H. Chang, and A. Agarwal, “Heme oxygenase and the kidney,” *DNA and Cell Biology*, vol. 21, no. 4, pp. 307–321, 2002.
 - [23] P. A. Dennery, G. Visner, Y. H. Weng et al., “Resistance to hyperoxia with heme oxygenase-1 disruption: role of iron,” *Free Radical Biology and Medicine*, vol. 34, no. 1, pp. 124–133, 2003.
 - [24] R. Öllinger, H. Wang, K. Yamashita et al., “Therapeutic applications of bilirubin and biliverdin in transplantation,” *Antioxidants and Redox Signaling*, vol. 9, no. 12, pp. 2175–2185, 2007.
 - [25] L. Brasile, R. Buelow, B. M. Stubenitsky, and G. Kootstra, “Induction of heme oxygenase-1 in kidneys during ex vivo warm perfusion,” *Transplantation*, vol. 76, no. 8, pp. 1145–1149, 2003.
 - [26] D. Willis, A. R. Moore, R. Frederick, and D. A. Willoughby, “Heme oxygenase: a novel target for the modulation of the inflammatory response,” *Nature Medicine*, vol. 2, no. 1, pp. 87–90, 1996.
 - [27] K. D. Poss and S. Tonegawa, “Heme oxygenase 1 is required for mammalian iron reutilization,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 20, pp. 10919–10924, 1997.
 - [28] N. Radhakrishnan, S. P. Yadav, A. Sachdeva et al., “Human heme oxygenase-1 deficiency presenting with hemolysis, nephritis, and asplenia,” *Journal of Pediatric Hematology/Oncology*, vol. 33, no. 1, pp. 74–78, 2011.
 - [29] C. Fondevila, X. D. Shen, S. Tsuchiyashi et al., “Biliverdin therapy protects rat livers from ischemia and reperfusion injury,” *Hepatology*, vol. 40, no. 6, pp. 1333–1341, 2004.
 - [30] Y. Liu, B. Zhu, X. Wang et al., “Bilirubin as a potent antioxidant suppresses experimental autoimmune encephalomyelitis: implications for the role of oxidative stress in the development of multiple sclerosis,” *Journal of Neuroimmunology*, vol. 139, no. 1–2, pp. 27–35, 2003.
 - [31] B. A. Moore, L. E. Otterbein, A. Türler, A. M. K. Choi, and A. J. Bauer, “Inhaled carbon monoxide suppresses the development of postoperative ileus in the murine small intestine,” *Gastroenterology*, vol. 124, no. 2, pp. 377–391, 2003.
 - [32] D. J. Slebos, S. W. Ryter, and A. M. K. Choi, “Heme oxygenase-1 and carbon monoxide in pulmonary medicine,” *Respiratory Research*, vol. 4, article 7, 2003.
 - [33] A. M. Davalli, L. Scaglia, D. H. Zangen, J. Hollister, S. Bonner-Weir, and G. C. Weir, “Vulnerability of islets in the immediate posttransplantation period: dynamic changes in structure and function,” *Diabetes*, vol. 45, no. 9, pp. 1161–1167, 1996.
 - [34] Y. X. Li, G. Li, W. P. Dong, D. R. Lu, and J. M. Tan, “Protection of human islets from induction of apoptosis and improved islet function with HO-1 gene transduction,” *Chinese Medical Journal*, vol. 119, no. 19, pp. 1639–1645, 2006.
 - [35] B. R. S. Hsu, J. H. Juang, S. T. Chen, S. Hsu, and S. H. Fu, “Cobalt-protoporphyrin treatment renders islets tolerant to interleukin-1 beta suppression,” *Transplantation Proceedings*, vol. 36, no. 4, pp. 1181–1182, 2004.
 - [36] A. Pileggi, R. Damaris Molano, T. Berney et al., “Heme oxygenase-1 induction in islet cells results in protection from apoptosis and improved *in vivo* function after transplantation,” *Diabetes*, vol. 50, no. 9, pp. 1983–1991, 2001.
 - [37] M. M. Ribeiro, D. Klein, A. Pileggi et al., “Heme oxygenase-1 fused to a TAT peptide transduces and protects pancreatic β -cells,” *Biochemical and Biophysical Research Communications*, vol. 305, no. 4, pp. 876–881, 2003.
 - [38] A. Pileggi, R. D. Molano, T. Berney et al., “Prolonged allogeneic islet graft survival by protoporphyrins,” *Cell Transplantation*, vol. 14, no. 2–3, pp. 85–96, 2005.
 - [39] S. H. Fu, B. R. S. Hsu, J. H. Juang, S. T. Chen, T. Y. Yang, and S. Hsu, “Cobalt-protoporphyrin treatment enhances murine islets engraftment,” *Transplantation Proceedings*, vol. 36, no. 7, pp. 2205–2206, 2004.
 - [40] H. Wang, S. S. Lee, W. Gao et al., “Donor treatment with carbon monoxide can yield islet allograft survival and tolerance,” *Diabetes*, vol. 54, no. 5, pp. 1400–1406, 2005.
 - [41] A. Goldberg, M. Parolini, B. Y. Chin et al., “Toll-like receptor 4 suppression leads to islet allograft survival,” *FASEB Journal*, vol. 21, no. 11, pp. 2840–2848, 2007.
 - [42] S. Bertera, M. L. Crawford, A. M. Alexander et al., “Gene transfer of manganese superoxide dismutase extends islet graft function in a mouse model of autoimmune diabetes,” *Diabetes*, vol. 52, no. 2, pp. 387–393, 2003.
 - [43] B. Wegiel, C. J. Baty, D. Gallo et al., “Cell surface biliverdin reductase mediates biliverdin-induced anti-inflammatory effects via phosphatidylinositol 3-kinase and Akt,” *Journal of Biological Chemistry*, vol. 284, no. 32, pp. 21369–21378, 2009.

- [44] C. G. Thanos and R. B. Elliott, "Encapsulated porcine islet transplantation: an evolving therapy for the treatment of Type I diabetes," *Expert Opinion on Biological Therapy*, vol. 9, no. 1, pp. 29–44, 2009.
- [45] D. Dufrane and P. Gianello, "Pig islets for clinical islet xenotransplantation," *Current Opinion in Nephrology and Hypertension*, vol. 18, no. 6, pp. 495–500, 2009.
- [46] D. Dufrane and P. Gianello, "Pig islet xenotransplantation into non-human primate model," *Transplantation*, vol. 86, no. 6, pp. 753–760, 2008.
- [47] S. J. Chen, Y. L. Wang, W. T. Lo et al., "Erythropoietin enhances endogenous haem oxygenase-1 and represses immune responses to ameliorate experimental autoimmune encephalomyelitis," *Clinical and Experimental Immunology*, vol. 162, no. 2, pp. 210–223, 2010.
- [48] X. Chen, Z. Zhang, C. Su, W. Gu, H. Li, and G. Zhou, "Protective effect of heme oxygenase-1 to pancreas islet xenograft," *Journal of Surgical Research*, vol. 164, pp. 336–343, 2010.
- [49] C. Su, X. Chen, Z. Y. Zhang et al., "Protective effect of heme oxygenase-1 induction *in vivo* to pancreas islet xenograft," *Zhonghua Wai Ke Za Zhi*, vol. 47, no. 16, pp. 1249–1252, 2009.
- [50] S. M. Opal, J. C. Wherry, and P. Grint, "Interleukin-10: potential benefits and possible risks in clinical infectious diseases," *Clinical Infectious Diseases*, vol. 27, no. 6, pp. 1497–1507, 1998.
- [51] M. Battaglia, C. Gianfrani, S. Gregori, and M. G. Roncarolo, "IL-10-producing T regulatory type 1 cells and oral tolerance," *Annals of the New York Academy of Sciences*, vol. 1029, pp. 142–153, 2004.
- [52] H. Toyoda and B. Formby, "Contribution of T cells to the development of autoimmune diabetes in the NOD mouse model," *BioEssays*, vol. 20, no. 9, pp. 750–757, 1998.
- [53] M. O. Kurrer, S. V. Pakala, H. L. Hanson, and J. D. Katz, "β cell apoptosis in T cell-mediated autoimmune diabetes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 1, pp. 213–218, 1997.
- [54] A. Rabinovitch and W. L. Suarez-Pinzon, "Cytokines and their roles in pancreatic islet β-cell destruction and insulin-dependent diabetes mellitus," *Biochemical Pharmacology*, vol. 55, no. 8, pp. 1139–1149, 1998.
- [55] E. Horio, M. Fukuda, H. Katoh et al., "Reactive oxygen intermediates in autoimmune islet cell destruction of the NOD mouse induced by peritoneal exudate cells (rich in macrophages) but not T cells," *Diabetologia*, vol. 37, no. 1, pp. 22–31, 1994.
- [56] M. Li, S. Peterson, D. Husney et al., "Interdiction of the diabetic state in NOD mice by sustained induction of heme oxygenase: possible role of carbon monoxide and bilirubin," *Antioxidants and Redox Signaling*, vol. 9, no. 7, pp. 855–863, 2007.
- [57] C. M. Hu, H. H. Lin, M. T. Chiang, P. F. Chang, and L. Y. Chau, "Systemic expression of heme oxygenase-1 ameliorates type 1 diabetes in NOD mice," *Diabetes*, vol. 56, no. 5, pp. 1240–1247, 2007.
- [58] S. H. Huang, C. H. Chu, J. C. Yu et al., "Transgenic expression of haem oxygenase-1 in pancreatic beta cells protects non-obese mice used as a model of diabetes from autoimmune destruction and prolongs graft survival following islet transplantation," *Diabetologia*, vol. 53, no. 11, pp. 2389–2400, 2010.
- [59] D. J. Park, A. Agarwal, and J. F. George, "Heme oxygenase-1 expression in murine dendritic cell subpopulations: effect on CD8⁺ dendritic cell differentiation *in vivo*," *American Journal of Pathology*, vol. 176, no. 6, pp. 2831–2839, 2010.
- [60] R. Henningsson, P. Alm, P. Ekström, and I. Lundquist, "Heme oxygenase and carbon monoxide: regulatory roles in islet hormone release: a biochemical, immunohistochemical, and confocal microscopic study," *Diabetes*, vol. 48, no. 1, pp. 66–76, 1999.
- [61] M. Li, D. H. Kim, P. L. Tsenovoy et al., "Treatment of obese diabetic mice with a heme oxygenase inducer reduces visceral and subcutaneous adiposity, increases adiponectin levels, and improves insulin sensitivity and glucose tolerance," *Diabetes*, vol. 57, no. 6, pp. 1526–1535, 2008.
- [62] C. R. Bruce, A. L. Carey, J. A. Hawley, and M. A. Febbraio, "Intramuscular heat shock protein 72 and heme oxygenase-1 mRNA are reduced in patients with type 2 diabetes: evidence that insulin resistance is associated with a disturbed antioxidant defense mechanism," *Diabetes*, vol. 52, no. 9, pp. 2338–2345, 2003.
- [63] J. F. Ndisang, N. Lane, and A. Jadhav, "The heme oxygenase system abates hyperglycemia in Zucker diabetic fatty rats by potentiating insulin-sensitizing pathways," *Endocrinology*, vol. 150, no. 5, pp. 2098–2108, 2009.
- [64] L. Rodella, B. D. Lamon, R. Rezzani et al., "Carbon monoxide and biliverdin prevent endothelial cell sloughing in rats with type I diabetes," *Free Radical Biology and Medicine*, vol. 40, no. 12, pp. 2198–2205, 2006.
- [65] J. F. Ndisang and A. Jadhav, "Heme oxygenase system enhances insulin sensitivity and glucose metabolism in streptozotocin-induced diabetes," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 296, no. 4, pp. E829–E841, 2009.
- [66] Y. E. Jing and S. G. Laychock, "A protective role for heme oxygenase expression in pancreatic islets exposed to interleukin-1β," *Endocrinology*, vol. 139, no. 10, pp. 4155–4163, 1998.
- [67] M. A. Di Noia, S. Van Driesche, F. Palmieri et al., "Heme oxygenase-1 enhances renal mitochondrial transport carriers and cytochrome C oxidase activity in experimental diabetes," *Journal of Biological Chemistry*, vol. 281, no. 23, pp. 15687–15693, 2006.
- [68] M. Di Pascoli, L. Rodella, D. Sacerdoti, M. Bolognesi, S. Turkseven, and N. G. Abraham, "Chronic CO levels has a beneficial effect on vascular relaxation in diabetes," *Biochemical and Biophysical Research Communications*, vol. 340, no. 3, pp. 935–943, 2006.
- [69] I. Lundquist, P. Alm, A. Salehi, R. Henningsson, E. Grapengiesser, and B. Hellman, "Carbon monoxide stimulates insulin release and propagates Ca²⁺ signals between pancreatic β-cells," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 285, no. 5, pp. E1055–E1063, 2003.
- [70] V. M. Dixit, S. Green, V. Sarma et al., "Tumor necrosis factor-α induction of novel gene products in human endothelial cells including a macrophage-specific chemotaxin," *Journal of Biological Chemistry*, vol. 265, no. 5, pp. 2973–2978, 1990.
- [71] Y. C. Liu, J. Penninger, and M. Karin, "Immunity by ubiquitylation: a reversible process of modification," *Nature Reviews Immunology*, vol. 5, no. 12, pp. 941–952, 2005.
- [72] H. Y. Song, M. Rothe, and D. V. Goeddel, "The tumor necrosis factor-inducible zinc finger protein A20 interacts with TRAF1/TRAF2 and inhibits NF-κB activation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 13, pp. 6721–6725, 1996.

- [73] J. T. Cooper, D. M. Stroka, C. Brostjan, A. Palmethofer, F. H. Bach, and C. Ferran, "A20 blocks endothelial cell activation through a NF- κ B-dependent mechanism," *Journal of Biological Chemistry*, vol. 271, no. 30, pp. 18068–18073, 1996.
- [74] H. W. Chng, R. S. Camplejohn, M. G. Stone, I. R. Hart, and L. J. Nicholson, "A new role for the anti-apoptotic gene A20 in angiogenesis," *Experimental Cell Research*, vol. 312, no. 15, pp. 2897–2907, 2006.
- [75] S. T. Grey, M. B. Arvelo, W. Hasenkamp, F. H. Bach, and C. Ferran, "A20 inhibits cytokine-induced apoptosis and nuclear factor κ B-dependent gene activation in islets," *Journal of Experimental Medicine*, vol. 190, no. 8, pp. 1135–1145, 1999.
- [76] S. T. Grey, C. Longo, T. Shukri et al., "Genetic engineering of a suboptimal islet graft with A20 preserves β cell mass and function," *Journal of Immunology*, vol. 170, no. 12, pp. 6250–6256, 2003.
- [77] D. Liuwantara, M. Elliot, M. W. Smith et al., "Nuclear factor-kappaB regulates β -cell death: a critical role for A20 in β -cell protection," *Diabetes*, vol. 55, no. 9, pp. 2491–2501, 2006.
- [78] T. Miralem, Z. Hu, M. D. Torno, K. M. Lelli, and M. D. Maines, "Small interference RNA-mediated gene silencing of human biliverdin reductase, but not that of heme oxygenase-1, attenuates arsenite-mediated induction of the oxygenase and increases apoptosis in 293A kidney cells," *Journal of Biological Chemistry*, vol. 280, no. 17, pp. 17084–17092, 2005.
- [79] N. Lerner-Marmarosh, J. Shen, M. D. Torno, A. Kravets, Z. Hu, and M. D. Maines, "Human biliverdin reductase: a member of the insulin receptor substrate family with serine/threonine/tyrosine kinase activity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 20, pp. 7109–7114, 2005.
- [80] D. J. van der Windt, R. Bottino, A. Casu, N. Campanile, and D. K. C. Cooper, "Rapid loss of intraportally transplanted islets: an overview of pathophysiology and preventive strategies," *Xenotransplantation*, vol. 14, no. 4, pp. 288–297, 2007.
- [81] A. Jackson, C. McWilliams, E. Kaizer et al., "Gene expression profiling of human pancreatic islets undergoing a simulated process of instant blood-mediated inflammatory reaction," *Transplantation Proceedings*, vol. 40, no. 2, pp. 430–432, 2008.
- [82] H. Zhu, J. Wang, H. Jiang et al., "Bilirubin protects grafts against nonspecific inflammation-induced injury in syngeneic intraportal islet transplantation," *Experimental and Molecular Medicine*, vol. 42, no. 11, pp. 739–748, 2010.
- [83] H. Wang, S. L. Soo, C. Dell'Agnello et al., "Bilirubin can induce tolerance to islet allografts," *Endocrinology*, vol. 147, no. 2, pp. 762–768, 2006.
- [84] S. S. Lee, W. Gao, S. Mazzola et al., "Heme oxygenase-1, carbon monoxide, and bilirubin induce tolerance in recipients toward islet allografts by modulating T regulatory cells," *FASEB Journal*, vol. 21, no. 13, pp. 3450–3457, 2007.
- [85] Y. Y. Fu, K. J. Kang, J. M. Ahn et al., "Hyperbilirubinemia reduces the streptozotocin-induced pancreatic damage through attenuating the oxidative stress in the Gunn rat," *Tohoku Journal of Experimental Medicine*, vol. 222, no. 4, pp. 265–273, 2010.
- [86] F. Rocuts, X. Zhang, J. Yan et al., "Bilirubin promotes de novo generation of T regulatory cells," *Cell Transplantation*, vol. 19, no. 4, pp. 443–451, 2010.
- [87] A. Nakao, J. S. Neto, S. Kanno et al., "Protection against ischemia/reperfusion injury in cardiac and renal transplantation with carbon monoxide, biliverdin and both," *American Journal of Transplantation*, vol. 5, no. 2, pp. 282–291, 2005.
- [88] N. Ikeda, T. Inoguchi, N. Sonoda et al., "Biliverdin protects against the deterioration of glucose tolerance in db/db mice," *Diabetologia*, vol. 54, no. 8, pp. 2183–2191, 2011.
- [89] R. Mancarella, S. Del Guerra, M. Masini et al., "Beneficial effect of the nonpeptidyl low molecular weight radical scavenger IAC on cultured human islet function," *Cell Transplantation*, vol. 17, no. 10-11, pp. 1271–1276, 2008.
- [90] J. Qin, Y. Jiao, X. Chen, S. Zhou, C. Liang, and C. Zhong, "Overexpression of suppressor of cytokine signaling 1 in islet grafts results in anti-apoptotic effects and prolongs graft survival," *Life Sciences*, vol. 84, no. 23-24, pp. 810–816, 2009.
- [91] F. C. Chou and H. K. Sytwu, "Overexpression of thioredoxin in islets transduced by a lentiviral vector prolongs graft survival in autoimmune diabetic NOD mice," *Journal of Biomedical Science*, vol. 16, no. 1, article 71, 2009.
- [92] B. Zhang, M. Kang, Q. Xie et al., "Anthocyanins from chinese bayberry extract protect β cells from oxidative stress-mediated injury via HO-1 upregulation," *Journal of Agricultural and Food Chemistry*, vol. 59, no. 2, pp. 537–545, 2011.
- [93] J. Emamaullee, P. Liston, R. G. Korneluk, A. M. J. Shapiro, and J. F. Elliott, "XIAP overexpression in islet β -cells enhances engraftment and minimizes hypoxia-reperfusion injury," *American Journal of Transplantation*, vol. 5, no. 6, pp. 1297–1305, 2005.
- [94] J. A. Emamaullee, R. V. Rajotte, P. Liston et al., "XIAP overexpression in human islets prevents early posttransplant apoptosis and reduces the islet mass needed to treat diabetes," *Diabetes*, vol. 54, no. 9, pp. 2541–2548, 2005.
- [95] J. A. Emamaullee, A. M. J. Shapiro, R. V. Rajotte, G. Korbutt, and J. F. Elliott, "Neonatal porcine islets exhibit natural resistance to hypoxia-induced apoptosis," *Transplantation*, vol. 82, no. 7, pp. 945–952, 2006.
- [96] H. Hui, N. Khoury, X. Zhao et al., "Adenovirus-mediated XIAP gene transfer reverses the negative effects of immunosuppressive drugs on insulin secretion and cell viability of isolated human islets," *Diabetes*, vol. 54, no. 2, pp. 424–433, 2005.
- [97] S. K. Hun, S. Kim, and M. S. Lee, "IFN- γ sensitizes MIN6N8 insulinoma cells to TNF- α -induced apoptosis by inhibiting NF- κ B-mediated XIAP upregulation," *Biochemical and Biophysical Research Communications*, vol. 336, no. 3, pp. 847–853, 2005.
- [98] K. A. Kim and M. S. Lee, "Recent progress in research on beta-cell apoptosis by cytokines," *Frontiers in Bioscience*, vol. 14, pp. 657–664, 2009.
- [99] D. Ou, X. Wang, D. L. Metzger et al., "Synergistic inhibition of tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in human pancreatic β cells by Bcl-2 and X-linked inhibitor of apoptosis," *Human Immunology*, vol. 66, no. 3, pp. 274–284, 2005.
- [100] A. Plesner, P. Liston, R. Tan, R. G. Korneluk, and C. B. Verchere, "The X-linked inhibitor of apoptosis protein enhances survival of murine islet allografts," *Diabetes*, vol. 54, no. 9, pp. 2533–2540, 2005.
- [101] A. Plesner, G. Soukhatcheva, R. G. Korneluk, and C. B. Verchere, "XIAP inhibition of β -cell apoptosis reduces the number of islets required to restore euglycemia in a syngeneic islet transplantation model," *Islets*, vol. 2, no. 1, pp. 18–23, 2010.
- [102] J. D. Rivas-Carrillo, A. Soto-Gutierrez, N. Navarro-Alvarez et al., "Cell-permeable pentapeptide V5 inhibits apoptosis and enhances insulin secretion, allowing experimental single-donor islet transplantation in mice," *Diabetes*, vol. 56, no. 5, pp. 1259–1267, 2007.

- [103] M. A. Robbins, L. Maksumova, E. Pocock, and J. K. Chantler, "Nuclear factor- κ B translocation mediates double-stranded ribonucleic acid-induced NIT-1 β -cell apoptosis and up-regulates caspase-12 and tumor necrosis factor receptor-associated ligand (TRAIL)," *Endocrinology*, vol. 144, no. 10, pp. 4616–4625, 2003.
- [104] H. Wu, R. Panakanti, F. Li, and R. I. Mahato, "XIAP gene expression protects β -cells and human islets from apoptotic cell death," *Molecular Pharmaceutics*, vol. 7, no. 5, pp. 1655–1666, 2010.
- [105] M. A. Atkinson, J. A. Bluestone, G. S. Eisenbarth et al., "How does type 1 diabetes develop? The notion of homicide or β -cell suicide revisited," *Diabetes*, vol. 60, no. 5, pp. 1370–1379, 2011.

Review Article

Striated Muscle as Implantation Site for Transplanted Pancreatic Islets

Daniel Espes,¹ Olof Eriksson,² Joey Lau,¹ and Per-Ola Carlsson^{1,3}

¹ Department of Medical Cell Biology, Husargatan 3, P.O. Box 571, Uppsala University, 75123 Uppsala, Sweden

² Preclinical PET Platform, Department of Medicinal Chemistry, Uppsala University, 75123 Uppsala, Sweden

³ Department of Medical Sciences, Uppsala University, 75185 Uppsala, Sweden

Correspondence should be addressed to Per-Ola Carlsson, per-ola.carlsson@medcellbiol.uu.se

Received 27 May 2011; Accepted 20 September 2011

Academic Editor: Antonello Pileggi

Copyright © 2011 Daniel Espes et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Islet transplantation is an attractive treatment for selected patients with brittle type 1 diabetes. In the clinical setting, intraportal transplantation predominates. However, due to extensive early islet cell death, the quantity of islets needed to restore glucose homeostasis requires in general a minimum of two donors. Moreover, the deterioration of islet function over time results in few insulin-independent patients after five-year followup. Specific obstacles to the success of islet transplantation include site-specific concerns for the liver such as the instant blood mediated inflammatory reaction, islet lipotoxicity, low oxygen tension, and poor revascularization, impediments that have led to the developing interest for alternative implantation sites over recent years. Within preclinical settings, several alternative sites have now been investigated and proven favorable in various aspects. Muscle is considered a very promising site and has physiological properties and technical advantages that could make it optimal for islet transplantation.

1. Introduction

Type 1 diabetes mellitus is a chronic disease with typical onset during childhood or adolescence. Patients suffering from the disease require multiple daily insulin injections coordinated alongside vigilant monitoring of blood glucose levels. For some patients, glucose homeostasis is not optimized despite these efforts, leading to repeated hyperglycemic and hypoglycemic events. Such repeated episodes of severe hypoglycemia, requiring medical assistance, predicate a patient being considered for islet transplantation. Currently, intraportal transplantation is performed in the majority of clinical cases. The liver was selected as the optimal implantation site for islet transplantation in the beginning of the 1970s, the basis of experimental studies by Kemp et al. [1]. However, clinical results of islet transplantation were meager with less than 10% of transplant recipients displaying insulin independence. The introduction of the Edmonton-protocol ten years ago drastically improved the outcome to a rate of 80% insulin independence after one year [2]. Nevertheless, this

procedure requires the islets of multiple donors and provides inadequate long-term results. Only 10% of transplant recipients have maintained insulin-independency after five years despite continuing efforts [3]. Fundamentally, however, intraportal transplantation must be considered successful. The main stimulus for islet transplantation, repeat episodes of severe hypoglycemia, is prevented in most patients irrespective of insulin independence after transplantation and results in a marked increase in the quality of life.

During recent years, interest in alternative implantation sites with the potential to improve long-term results has produced advances in noninvasive methodologies towards the quantification of islet graft mass and function. Positron emission tomography (PET) is one such technique, providing a functional imaging modality that quantifies an in vivo distribution of bioactive compounds labeled with positron-emitting nuclides. Commonly, clinically used PET tracers are labeled with short-lived radionuclides that result in a radiation dose that is both localized and nontoxic, allowing for multiple scans of a single patient.

2. Obstacles with Intraportal Islet Transplantation

The rapid islet cell death observed in intraportal transplantation is in part caused by an instant blood-mediated inflammatory response (IBMIR) [4, 5]. The IBMIR consists of both thrombotic and complement activation cascades which lead to the formation of blood clots around the islets, leucocyte infiltration, and disruption of islet morphology [6]. In clinical settings, the IBMIR can be detected immediately after islet infusion as an increase in both thrombin-antithrombin complexes and increased c-peptide levels due to beta-cell disruption and death [5]. With a combined positron emission tomography and computed tomography (PET/CT) technique, the early cell death after intraportal islet transplantation has been estimated at approximately 25% [7].

In conjunction to the IBMIR, characteristic hypoxic events have also been contributed to early islet cell death. Indeed, during the process of initial islet isolation, the native vascular connections are disrupted, enforcing the islets to depend on oxygen diffusion immediately after transplantation. This process is further complicated when considering that the liver parenchyma has a much lower oxygen tension than can be found in the pancreas [8]. We recently observed in an experimental animal model that approximately 70% of islets are hypoxic one day after intraportal transplantation [9]. This finding was accomplished employing pimonidazole, a biochemical marker that accumulates in islet cells with oxygen tension levels below 7.5–10 mmHg. Notably, the hypoxia may even be underestimated in these experiments, as pimonidazole adducts are not incorporated within dead or dying cells. In addition, our team has also observed that caspase-3 staining of islets reveals an apoptosis level, correlating to the hypoxia, as high as 10% in islets one day after intraportal transplantation [10].

It has been proposed that the high concentrations of immunosuppressive drugs in the portal vein, following intestinal uptake of the drugs, may have a toxic effect on transplanted islet cells in the liver [11, 12]. Moreover, several long-term liver-site-specific challenges have been identified such as lipotoxicity to the transplanted cells [13, 14] and insufficient vascular engraftment [15, 16]. Intrahepatically transplanted islets are slowly revascularized, severely impairing blood perfusion and oxygenation up to three months after transplantation [17]. Considering the aforementioned challenges, substantial hurdles remain to be overcome to improve the long-term prognosis of existing intraportal islet transplantation procedures.

3. Considerations for a New Implantation Site

Over the last decade, there has been an increased interest in alternative implantation sites due to the identified obstacles and the deterioration of islet graft function after time following intraportal transplantation. Several factors must be considered when choosing an optimal implantation site. The prospective organ requires physiological characteristics that are favorable for islets in regards to oxygen tension, vascular

supply, and angiogenesis. Implanted beta cells must also have the capacity to adapt to the implantation organ and maintain their differentiation. Furthermore, a viable route for insulin secretion and glycemic detection is required. Some sites, such as the testis, thymus, brain, and eye are immune-privileged making them tempting candidates. Considering the technical and surgical aspects, an ideal site should have easy access, allowing for a minimally invasive procedure. Preferably, it should be possible to obtain biopsies without causing undue risk to either the patient or the graft. There is current optimism towards developing methods able to quantify the islet cell mass with imaging techniques. Several PET probes intended for noninvasive visualization of endogenous beta cells in the pancreas have been investigated during recent years, generally targeting the monoamine pathways as these are absent in exocrine tissue which compose the vast majority (>95%) of the pancreatic volume. PET tracers have been utilized for studies of the central nervous system (CNS) since the 1980s, in particular for the serotonergic and dopaminergic systems, although various tracers have now been applied to image endogenous beta cells. These include carbon-11, fluorine-18, or gallium-68 labeled versions of small molecule ligands or peptides, targeting vesicular monoamine transport [18] and oxidation as well as serotonin and dopamine synthesis [19, 20]. Other recently available PET tracers act as ligands for beta cell-specific markers such as sulfonylurea receptor-1 [21] and glucagon-like peptide-1 receptor [22]. It has also been suggested that early beta cell destruction due to autoimmunity or rejection can be studied by [¹⁸F]FDG, a marker for glucose metabolism, imaging both inflammatory lesions and insulinitis [23]. Technical advances which image endogenous beta cell mass can naturally be applied in islet transplantation studies.

The pancreas is a tempting implantation site. It is the natural milieu for islets. In experimental studies we have shown that the vascular density in islets transplanted to the pancreas is only slightly lower than in endogenous islets [24]. We have also shown that in contrast to intrahepatically transplanted islets, islets transplanted to the pancreas display less severe changes in gene expression and have only moderate changes in their metabolic function posttransplantation [25]. However, injection into the pancreas may elicit leakage of exocrine enzymes, resulting in pancreatitis. Therefore, the pancreas has rarely been considered as a clinically feasible implantation site. Improved implantation techniques, for example, beneath the pancreatic capsule, as well as safety and efficacy studies in large animal models are clearly needed before considering clinical trials.

Superior islet survival has recently been demonstrated following experimental transplantation into the omentum when compared to intraportal islet transplantation [26]. Since the 1980s, the omental pouch has been considered as a potential site for islet transplantation [27]. A primate model study revealed an observable delay in transplant engraftment when compared to intraportal transplantation, yet over time, recipients obtained equitable plasma C-peptide levels [28]. Although the vascular engraftment of islets in omentum is delayed, the omentum or omental pouch has the capacity to be a useful clinical site.

The anterior chamber of the eye provides excellent conditions for engraftment of transplanted islets [29]. Injected islets engraft on the iris become fully revascularized and have the capacity to reverse diabetes in mice [29]. A recent follow-up study in diabetic baboons also showed successful long-term survival and function of islets after allogeneic transplantation [30]. Furthermore, transplanted islets contributed to glycemic control without any perceived impairment of vision. The implantation procedure was minimally invasive, and considering the immune-privileged properties of this site, it can be concluded as an attractive site for clinical use. However, pertinent safety and efficacy studies remain, bearing in mind the potential impact on transplant recipients visual function if transplanting islets of a sufficient magnitude to reverse hyperglycemia.

4. Intramuscular Islet Transplantation: A Promising Implantation Site

Striated muscle has been used for decades as a site for autologous transplantation of normal parathyroid tissue in patients with hyperparathyroidism, with excellent results [31]. Such grafts have proven highly functional for more than 10 years, with full restoration of calcium homeostasis [31]. The procedure is well documented with few complications and is considered a standard technique.

Muscle has a naturally occurring angiogenesis with a higher oxygen tension than can be found in the liver parenchyma. We have recently shown that there is a rapid revascularization of intramuscularly transplanted mouse and human islets, containing a blood vessel incidence on par with native islets within two weeks posttransplantation [32]. Graft blood perfusion was restored and the oxygen tension of intramuscular islets only slightly diminished compared to native islets after revascularization [33]. This paradigm is further supported in the clinical setting and witnessed through magnetic resonance imaging of pancreatectomized patients receiving autotransplantation of isolated islets to the brachioradialis muscle, where a high revascularization of intramuscular islet grafts has also been observed [32]. Experimentally, islets transplanted to muscle also have a superior glucose tolerance compared to recipients of similar numbers of islets transplanted intraportally [32]. A case report from the Nordic Network for Clinical Islet Transplantation, where a young patient with hereditary pancreatitis underwent total pancreatectomy treated by an autologous islet transplant to the brachioradialis muscle, also showed sustained insulin production from the graft for more than two years [34].

However, it is arguably the implantation technique itself that is pivotal to a high success rate at the intramuscular site. Injection of large clusters of islets causes substantial early islet cell death due to hypoxia. In such grafts, massive fibrosis can be observed both experimentally and clinically [32, 33] (Figure 1). This could explain the previous erratic success in experimental and clinical intramuscular islet transplantation [35–37]. It had become apparent to our group that in order to improve early islet survival, a shift in the transplantation procedure was required that disperses the islets throughout the tissue, for example, transplanting the islets along a “pearls

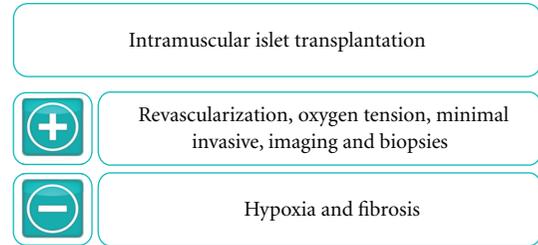


FIGURE 1: Summary of beneficial aspects and known obstacles for intramuscular islet transplantation.

on a string” fashion. Early cell death might be further reduced by remodeling the transplant site with bioengineered matrices and/or cotransplantation of oxygen carriers such as perfluorocarbons. There is also the possibility of improving the vascular network in the muscle prior to transplantation, since higher vascular density could easily be induced by angiogenic stimulators or hypoxia, both of which have a strong stimulatory effect on angiogenesis in muscle [38].

Due to minimal islet-blood contact within intramuscular transplantation, the challenge presented by the IBMIR is reduced. The capacity to transplant islets into a designated area in the muscle also facilitates site remodeling of immunological events prior to transplantation. Specifically, this could include modulation of early inflammation with mesenchymal stem cells, providing the potential to accelerate revascularization, as shown in both intraportal islet transplantation [39, 40] and islets transplanted beneath the kidney capsule [41]. Mesenchymal stem cells could either be cotransplanted or injected in advance to modulate the site.

It has earlier been reported that even moderate exercise causes hypoglycemia in rats with islets transplanted to the liver, kidney, and peritoneal cavity [42]. Hypoglycemic events can also be observed following intrapancreatic transplantation of islets [43]. Upregulation of lactate dehydrogenase in transplanted islets, with concomitantly increased lactate production eliciting insulin release during periods of low blood glucose levels, may be an explanation for this [25, 44]. It has not yet been reported how lactate dehydrogenase levels and other gene expression are affected in betacells of intramuscular islet grafts. Exercise might also cause a site-specific challenge to intramuscular islet grafts due to intramuscular pressure changes, since the glucose-consumption of muscle cells increases during exercise in accord with the regional blood flow.

5. Monitoring of Islets at the Intramuscular Site

It is difficult to obtain biopsies of islets transplanted into the liver. Islets are spread throughout the liver parenchyma, and liver biopsies *per se* are not free of risk. However, in the muscle, a biopsy would be simple to obtain. Preemptively dividing the transplanted islet mass, designating a specific islet mass to a separate site intended for potential biopsies, would allow subsequent studies to identify early markers for rejection.

The feasibility of imaging transplanted islets through PET techniques depends to a large extent on the site of im-

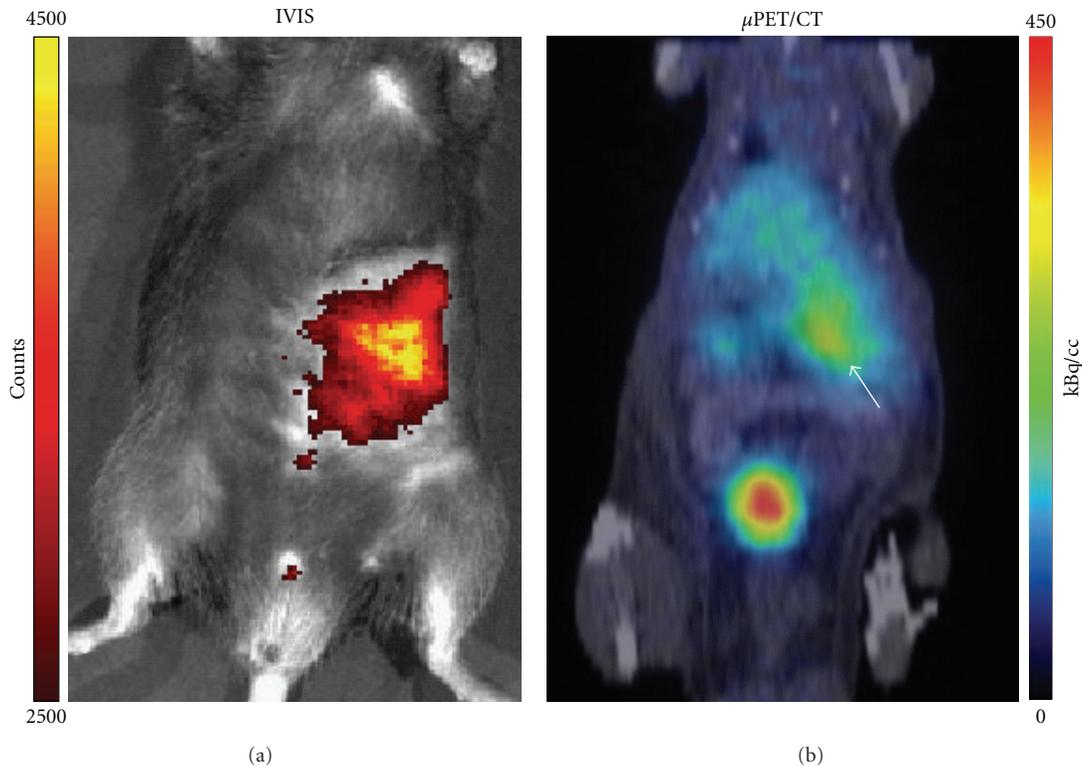


FIGURE 2: PET imaging of intramuscularly transplanted islets using the VMAT2 ligand [^{18}F]FE-DTBZ-d $_4$ as PET tracer. An inbred C57BL/6 mouse was transplanted with 300 mouse islets to the left abdominal muscle. The islets were labeled with Q-tracker prior to implantation. One month later, the mouse was administered the PET tracer [^{18}F]FE-DTBZ-d $_4$ intravenously. The tracer uptake could be detected by a μ PET/CT scanner after 60 minutes (right image, orange delineation). The corresponding location of the islets as determined by Q-tracker fluorescence emission is shown to the left.

plantation. This can be understood by examining the composition of a PET tracer signal emitted within a given region *in vivo*. In the main, a signal containing a composite of specific binding is produced (i), or the PET probe bound to the receptor of interest is proportional to receptor density. Displaying nonspecific binding (ii), the PET probe may also bind to other structures, revealing vascular contribution (iii). Vascular contribution is vital, as the tissue uptake of tracer is limited by local perfusion, potentiating an underestimation of uptake in grafts with low revascularization. All of these factors influence our ability to study islet grafts in different tissues.

With these issues in mind, we can see that longitudinal noninvasive visualization of hepatic grafts presents a considerable challenge, with substantial graft dilution (i). In addition, it is difficult to design PET tracers with a low hepatic background signal. Most tracers are metabolized in the liver (ii). The perfusion (iii), however, is sufficient to transport the tracer to engrafted islets in hepatic sinusoids shortly after transplantation.

This paradigm is reversed when considering intramuscular islet grafts. Grafts are generally pure and concentrated (i), with the above-mentioned PET tracers effecting a low to negligible uptake in muscle tissue (ii). Revascularization is therefore the limiting factor for visualization of the graft. It has recently been shown that intramuscular islet grafts attain a vascularization comparable to that in pancreatic islets after

approximately 2 weeks [32]. Considering these illustrations, quantification of intramuscular islet grafts containing an adequate volume is potentially achievable after engraftment and revascularization has been completed.

Progress with visualizing has been made both in preclinical and clinical studies of intrahepatic and intramuscularly transplanted islets using PET. The hepatic distribution and survival of islets during the peritransplant phase, following intraportal islet transplantation, has been studied with *ex vivo* labeling of islets by [^{18}F]FDG prior to infusion in murine [45] and porcine [46] models as well as in the clinic [7]. However, longitudinal studies are not yet possible using the *ex vivo* labeling methodology.

Uptake in an intramuscular islet graft of a vesicular monoamine transporter 2-(VMAT2-) ligand, [^{11}C]DTBZ, correlated well to the observable decrease in blood sugar of a preclinical STZ mouse model in a study by Witkowski et al. [47]. However, interpretation of the results and translation to the clinical situation is problematic when considering that the islets were implanted using a bioscaffold.

Pattou et al. [48] showed proof-of-principle in the clinic by visualizing islets transplanted to the brachioradialis muscle in a type 1 diabetic patient by intravenous administration of a [^{111}In]-labeled exendin-4, a GLP-1R ligand, using SPECT, an imaging modality related to PET.

The VMAT2 ligand [^{18}F]FE-DTBZ-d $_4$ (see Figure 2) and the catecholamine precursor [^{18}F]L-DOPA (unpublished data)

are currently being investigated as biomarkers for transplanted beta cells in two ongoing preclinical studies of intramuscularly transplanted islets in mice.

6. Conclusions

Striated muscle is a promising implantation site and in many regards superior to the liver. However, it is not yet fully characterized regarding islet long-term survival and functionality. The obstacles with early islet cell death observed to date following intramuscular islet transplantation can likely be overcome through remodeling the implantation site. Further studies capable of actualizing effective strategies are clearly needed, within both small and large animal models.

Acknowledgments

Own studies cited were supported by the Juvenile Diabetes Research Foundation, the Swedish Research Council, the EFSD/Novo Nordisk Programme 2010, the Swedish Diabetes Association, the Novo Nordisk Foundation, AFA Insurance the Swedish Juvenile Diabetes Fund, the Anér Foundation, the Thuring Foundation, the Goljes Memorial Fund, and the Family Ernfors Fund. J. Lau was supported by a postdoctoral scholarship from the Swedish Society for Medical Research. Beau Corazon is gratefully acknowledged for linguistic revision of this paper.

References

- [1] C. B. Kemp, M. J. Knight, and D. W. Scharp, "Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats," *Diabetologia*, vol. 9, no. 6, pp. 486–491, 1973.
- [2] A. M. J. Shapiro, J. R. T. Lakey, E. A. Ryan et al., "Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen," *The New England Journal of Medicine*, vol. 343, no. 4, pp. 230–238, 2000.
- [3] E. A. Ryan, B. W. Paty, P. A. Senior et al., "Five-year follow-up after clinical islet transplantation," *Diabetes*, vol. 54, no. 7, pp. 2060–2069, 2005.
- [4] W. Bennet, B. Sundberg, C. G. Groth et al., "Incompatibility between human blood and isolated islets of Langerhans: a finding with implications for clinical intraportal islet transplantation?" *Diabetes*, vol. 48, no. 10, pp. 1907–1914, 1999.
- [5] L. Moberg, H. Johansson, A. Lukinius et al., "Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation," *The Lancet*, vol. 360, no. 9350, pp. 2039–2045, 2002.
- [6] L. Moberg, "The role of the innate immunity in islet transplantation," *Upsala Journal of Medical Sciences*, vol. 110, no. 1, pp. 17–55, 2005.
- [7] O. Eriksson, T. Eich, A. Sundin et al., "Positron emission tomography in clinical islet transplantation," *The American Journal of Transplantation*, vol. 9, no. 12, pp. 2816–2824, 2009.
- [8] P. O. Carlsson, F. Palm, A. Andersson, and P. Liss, "Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site," *Diabetes*, vol. 50, no. 3, pp. 489–495, 2001.
- [9] R. Olsson, "The microvasculature of endogenous and transplanted pancreatic islets," in *Medical Cell Biology*, pp. 1–83, Uppsala University, Uppsala, Sweden, 2006.
- [10] R. Olsson, "Increased numbers of low-oxygenated pancreatic islets after intraportal islet transplantation," *Diabetes*, vol. 60, no. 9, pp. 2350–2353, 2011.
- [11] N. M. Desai, J. A. Goss, S. Deng et al., "Elevated portal vein drug levels of sirolimus and tacrolimus in islet transplant recipients: local immunosuppression or islet toxicity," *Transplantation*, vol. 76, no. 11, pp. 1623–1625, 2003.
- [12] A. M. Shapiro, H. L. Gallant, G. H. Er et al., "The portal immunosuppressive storm: relevance to islet transplantation?" *Therapeutic Drug Monitoring*, vol. 27, no. 1, pp. 35–37, 2005.
- [13] Y. Lee, M. Ravazzola, B. H. Park, Y. K. Bashmakov, L. Orci, and R. H. Unger, "Metabolic mechanisms of failure of intraportally transplanted pancreatic β -cells in rats: role of lipotoxicity and prevention by leptin," *Diabetes*, vol. 56, no. 9, pp. 2295–2301, 2007.
- [14] C. B. Leitao, K. Bernetti, T. Tharavanij et al., "Lipotoxicity and decreased islet graft survival," *Diabetes Care*, vol. 33, no. 3, pp. 658–660, 2010.
- [15] G. Mattsson, L. Jansson, and P. O. Carlsson, "Decreased vascular density in mouse pancreatic islets after transplantation," *Diabetes*, vol. 51, no. 5, pp. 1362–1366, 2002.
- [16] J. Lau and P. O. Carlsson, "Low revascularization of human islets when experimentally transplanted into the liver," *Transplantation*, vol. 87, no. 3, pp. 322–325, 2009.
- [17] J. Lau Börjesson, P.-O. Bergren, M. Köhler, and P.-O. Carlsson, "Pancreatic islets transplanted intraportally into the liver in mice have a substantially lower blood flow than native islets," *Diabetologia*, vol. 53, supplement 1, p. S60, 2010.
- [18] N. R. Simpson, F. Souza, P. Witkowski et al., "Visualizing pancreatic β -cell mass with [^{11}C]DTBZ," *Nuclear Medicine and Biology*, vol. 33, no. 7, pp. 855–864, 2006.
- [19] K. P. Koopmans, O. C. Neels, I. P. Kema et al., "Improved staging of patients with carcinoid and islet cell tumors with ^{18}F -dihydroxy-phenyl-alanine and ^{11}C -5-hydroxy-tryptophan positron emission tomography," *Journal of Clinical Oncology*, vol. 26, no. 9, pp. 1489–1495, 2008.
- [20] H. Minn, S. Kauhanen, M. Seppänen, and P. Nuutila, " ^{18}F -FDOPA: a multiple-target molecule," *Journal of Nuclear Medicine*, vol. 50, no. 12, pp. 1915–1918, 2009.
- [21] B. Wangler, S. Schneider, O. Thews et al., "Synthesis and evaluation of (S)-2-(2- [^{18}F]fluoroethoxy)-4- ([3-methyl-1-(2-piperidin-1-yl-phenyl)-butyl-carbamoyl]-methyl)-benzoic acid ([^{18}F]repaglinide): a promising radioligand for quantification of pancreatic β -cell mass with positron emission tomography (PET)," *Nuclear Medicine and Biology*, vol. 31, no. 5, pp. 639–647, 2004.
- [22] M. Brom, W. J. G. Oyen, L. Joosten, M. Gotthardt, and O. C. Boerman, " ^{68}Ga -labelled exendin-3, a new agent for the detection of Insulinomas with PET," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 37, no. 7, pp. 1345–1355, 2010.
- [23] T. Kalliokoski, P. Nuutila, K. A. Virtanen et al., "Pancreatic glucose uptake in vivo in men with newly diagnosed type 1 diabetes," *Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 5, pp. 1909–1914, 2008.
- [24] J. Lau, C. Kampf, G. Mattsson et al., "Beneficial role of pancreatic microenvironment for angiogenesis in transplanted pancreatic islets," *Cell Transplantation*, vol. 18, no. 1, pp. 23–30, 2009.
- [25] J. Lau, G. Mattsson, C. Carlsson et al., "Implantation site-dependent dysfunction of transplanted pancreatic islets," *Diabetes*, vol. 56, no. 6, pp. 1544–1550, 2007.
- [26] D. Jacobs-Tulleneers-Thevissen, K. Bartholomeus, K. Suenens et al., "Human islet cell implants in a nude rat model of

- diabetes survive better in omentum than in liver with a positive influence of beta cell number and purity," *Diabetologia*, vol. 53, no. 8, pp. 1690–1699, 2010.
- [27] C. J. Simeonovic, D. P. Dhall, J. D. Wilson, and K. J. Lafferty, "A comparative study of transplant sites for endocrine tissue transplantation in the pig," *The Australian Journal of Experimental Biology and Medical Science*, vol. 64, part 1, pp. 34–41, 1986.
- [28] D. M. Berman, J. J. O'Neil, L. C. Coffey et al., "Long-term survival of nonhuman primate islets implanted in an omental pouch on a biodegradable scaffold," *The American Journal of Transplantation*, vol. 9, no. 1, pp. 91–104, 2009.
- [29] S. Speier, D. Nyqvist, O. Cabrera et al., "Noninvasive in vivo imaging of pancreatic islet cell biology," *Nature Medicine*, vol. 14, no. 5, pp. 574–578, 2008.
- [30] V. L. Perez, A. Caicedo, D. M. Berman et al., "The anterior chamber of the eye as a clinical transplantation site for the treatment of diabetes: a study in a baboon model of diabetes," *Diabetologia*, vol. 54, no. 5, pp. 1121–1126, 2011.
- [31] Y. Tominaga, K. Uchida, T. Haba et al., "More than 1,000 cases of total parathyroidectomy with forearm autograft for renal hyperparathyroidism," *The American Journal of Kidney Diseases*, vol. 38, no. 4, supplement 1, pp. S168–S171, 2001.
- [32] G. Christoffersson, J. Henriksnäs, L. Johansson et al., "Clinical and experimental pancreatic islet transplantation to striated muscle: establishment of a vascular system similar to that in native islets," *Diabetes*, vol. 59, no. 10, pp. 2569–2578, 2010.
- [33] J. Svensson, J. Lau, M. Sandberg, and P.-O. Carlsson, "High vascular density and oxygenation of pancreatic islets transplanted in clusters into striated muscle," *Cell Transplantation*, vol. 20, no. 5, pp. 783–788, 2011.
- [34] E. Rafael, A. Tibell, M. Rydén et al., "Intramuscular autotransplantation of pancreatic islets in a 7-year-old child: a 2-year follow-up," *The American Journal of Transplantation*, vol. 8, no. 2, pp. 458–462, 2008.
- [35] K. V. Axen and F. X. Pi-Sunyer, "Long-term reversal of streptozotocin-induced diabetes in rats by intramuscular islet implantation," *Transplantation*, vol. 31, no. 6, pp. 439–441, 1981.
- [36] T. Lund, O. Korsgren, I. A. Aursnes, H. Scholz, and A. Foss, "Sustained reversal of diabetes following islet transplantation to striated musculature in the rat," *Journal of Surgical Research*, vol. 160, no. 1, pp. 145–154, 2010.
- [37] C. J. Weber, M. A. Hardy, and F. X. Pi Sunyer, "Tissue culture preservation and intramuscular transplantation of pancreatic islets," *Surgery*, vol. 84, no. 1, pp. 166–174, 1978.
- [38] B. M. Prior, H. T. Yang, and R. L. Terjung, "What makes vessels grow with exercise training?" *Journal of Applied Physiology*, vol. 97, no. 3, pp. 1119–1128, 2004.
- [39] T. Ito, S. Itakura, I. Todorov et al., "Mesenchymal stem cell and islet co-transplantation promotes graft revascularization and function," *Transplantation*, vol. 89, no. 12, pp. 1438–1445, 2010.
- [40] I. R. Duprez, U. Johansson, B. Nilsson, O. Korsgren, and P. U. Magnusson corresponding author, "Preparatory studies of composite mesenchymal stem cell islets for application in intraportal islet transplantation," *Uppsala Journal of Medical Sciences*, vol. 116, no. 1, pp. 8–17, 2011.
- [41] C. L. Rackham, P. C. Chagastelles, N. B. Nardi, A. C. Hauge-Evans, P. M. Jones, and A. J. King, "Co-transplantation of mesenchymal stem cells maintains islet organisation and morphology in mice," *Diabetologia*, vol. 54, no. 5, pp. 1127–1135, 2011.
- [42] A. Omer, V. F. Duvivier-Kali, W. Aschenbach, V. Tchipashvili, L. J. Goodyear, and G. C. Weir, "Exercise induces hypoglycemia in rats with islet transplantation," *Diabetes*, vol. 53, no. 2, pp. 360–365, 2004.
- [43] J. I. Stagner, S. P. Mokshagundam, and E. Samols, "Induction of mild hypoglycemia by islet transplantation to the pancreas," *Transplantation Proceedings*, vol. 30, no. 2, pp. 635–636, 1998.
- [44] P. O. Carlsson, A. Nordin, and F. Palm, "pH is decreased in transplanted rat pancreatic islets," *The American Journal of Physiology—Endocrinology and Metabolism*, vol. 284, no. 3, pp. E499–E504, 2003.
- [45] C. Toso, H. Zaidi, P. Morel et al., "Positron-emission tomography imaging of early events after transplantation of islets of langerhans," *Transplantation*, vol. 79, no. 3, pp. 353–355, 2005.
- [46] T. Eich, O. Eriksson, A. Sundin et al., "Positron emission tomography: a real-time tool to quantify early islet engraftment in a preclinical large animal model," *Transplantation*, vol. 84, no. 7, pp. 893–898, 2007.
- [47] P. Witkowski, H. Sondermeijer, M. A. Hardy et al., "Islet grafting and imaging in a bioengineered intramuscular space," *Transplantation*, vol. 88, no. 9, pp. 1065–1074, 2009.
- [48] F. Pattou, J. Kerr-Conte, and D. Wild, "GLP-1-receptor scanning for imaging of human beta cells transplanted in muscle," *The New England Journal of Medicine*, vol. 363, no. 13, pp. 1289–1290, 2010.

Review Article

Current Status of Immunomodulatory and Cellular Therapies in Preclinical and Clinical Islet Transplantation

Preeti Chhabra¹ and Kenneth L. Brayman^{1,2,3}

¹Department of Surgery, University of Virginia, Charlottesville, VA 22908, USA

²Division of Transplantation, Department of Surgery, University of Virginia, Charlottesville, VA 22908, USA

³The Center for Cellular Transplantation and Therapeutics, University of Virginia, Charlottesville, VA 22908, USA

Correspondence should be addressed to Kenneth L. Brayman, klb9r@hscmail.mcc.virginia.edu

Received 1 May 2011; Accepted 11 July 2011

Academic Editor: Antonello Pileggi

Copyright © 2011 P. Chhabra and K. L. Brayman. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Clinical islet transplantation is a β -cell replacement strategy that represents a possible definitive intervention for patients with type 1 diabetes, offering substantial benefits in terms of lowering daily insulin requirements and reducing incidences of debilitating hypoglycemic episodes and unawareness. Despite impressive advances in this field, a limiting supply of islets, inadequate means for preventing islet rejection, and the deleterious diabetogenic and nephrotoxic side effects associated with chronic immunosuppressive therapy preclude its wide-spread applicability. Islet transplantation however allows a window of opportunity for attempting various therapeutic manipulations of islets prior to transplantation aimed at achieving superior transplant outcomes. In this paper, we will focus on the current status of various immunosuppressive and cellular therapies that promote graft function and survival in preclinical and clinical islet transplantation with special emphasis on the tolerance-inducing capacity of regulatory T cells as well as the β -cells regenerative capacity of stem cells.

1. Introduction

Type 1 diabetes (T1D) is a T-cell-mediated autoimmune disease that involves the progressive destruction of pancreatic β cells resulting in complete loss of insulin secretion [1]. While a combination of genetic predisposition and autoimmune processes contributes to its development, inflammatory mediators and innate immunity play a key role in the induction and amplification of the immune assault as well as in the maintenance of insulinitis [2, 3]. Based on studies using animal models of autoimmune diabetes such as the biobreeding (BB) rat and the nonobese diabetic (NOD) mouse, the involvement of β -cell autoantigens, macrophages, dendritic cells (DCs), and T and B lymphocytes in the development of T1D has been established [4–6]. Antigen-presenting cells (APCs) such as macrophages and DCs are the first to infiltrate islets followed by CD4+ and CD8+ T lymphocytes, natural killer (NK) cells, and B lymphocytes. Macrophages play an essential role in the development and

activation of cytotoxic CD8+ T cells, the final effectors of β -cell destruction [5]. Studies indicate that IL-12 secreted by macrophages may activate Th1-type CD4+ T cells and subsequently IL-2 as well as other proinflammatory cytokines released by the activated CD4+ T cells (for e.g., interferon-gamma IFN- γ , tumor necrosis factor TNF- α and TNF- β) contribute to maximizing the activation of cytotoxic CD8+ T cells. Activated CD4+ and CD8+ T cells act in unison to activate β -cell death via apoptosis. Additionally, IFN- γ released by CD4+ T cells may in turn activate macrophages to release proinflammatory cytokines and reactive oxygen species (ROS, e.g., superoxide, hydrogen peroxide, nitric oxide (NO), etc.). Ultimately, T-cell-mediated β -cell destruction is effected overall by the interplay between receptor-mediated interactions (e.g., Fas-Fas ligand, CD40 ligand, TNF-TNF receptor), secretion of proinflammatory cytokines and ROS as well as the release of granzymes and perforin from cytotoxic effector T cells. Furthermore, DNA damage in β -cells leads to the activation of poly (ADP-ribose)

polymerase which rapidly depletes NAD that has been shown to be protective against radical-induced necrosis [7]. Interestingly, among the regulators of apoptosis, nuclear factor-kappa B (NF- κ B) has emerged as a master switch of cytokine-induced β -cell dysfunction and death [2]. Recognition of ligands by pattern-recognition receptors (PRRs, e.g., Toll-like receptor TLR3/4, RIGI, MDA5) on β -cells leads to the activation of key transcription factors such as NF- κ B and signal transducer and activator of transcription 1 (STAT-1), inducing the release of chemokines and cytokines that recruit and activate immune cells, increase expression of MHC class I antigens, and activate proapoptotic signals resulting in β -cell destruction. Proinflammatory cytokines further induce STAT-1, NF- κ B, and interferon regulatory factor 3 (IRF-3) in β -cells, contributing to the maintenance and amplification of the immune processes that result in the progressive and selective destruction of pancreatic β -cells [2].

Several studies indicate the potential role of cytokines in modulating the immune response towards cytotoxic effector cell or suppressor cell dominance. T1D is thought to be a Th1-dominance disease with type 1 cytokines such as IFN- γ , TNF- α , IL-2, IL-12, and IL-18 actively involved in β -cell destruction. On the other hand, cytokines such as IL-4, IL-5, IL-10 as well as TGF- β secreted by Th2/Th3 cells are thought to inhibit β -cell destruction. Of tremendous interest is the recent discovery that Th17 cells are potent inducers of tissue inflammation and autoimmunity and may have a role in T1D as indicated in a study using NOD mice [8, 9]. A humanized T1D model has now been established in NOD-scid IL2R γ (null) mice to study the selective destruction of mouse islet β -cells by a human T-cell-mediated immune response, providing a valuable tool for translational research into T1D [10].

Data from large epidemiologic studies indicate that T1D accounts for 5% to 10% of the total cases of diabetes worldwide, with its incidence increasing by 2% to 5%. In the United States, the prevalence of T1D by 18 years of age is approximately 1 in 300 [11, 12]. Strategies to prevent or reverse T1D are broadly based on the concepts of β -cell regeneration, β -cell replacement, or protection of islets from immune destruction. While regenerative strategies broadly involve β -cell regeneration from progenitors/stem cells (e.g., putative β -cell progenitors/pancreatic stem cells; embryonic, mesenchymal, hematopoietic, and umbilical cord blood-derived stem cells (UCB-SCs), induced pluripotent stem cells (iPSCs), etc.) or neogenesis from ductal and non- β -cell progenitors, β -cell replacement strategies include transplantation of whole pancreas or islets of Langerhans or genetically engineered insulin-secreting cells.

2. Islet Transplantation

The current standard of treatment for T1D requires lifelong exogenous insulin administration by either insulin pump or multiple daily injections which although successful by no means represents a cure, often resulting in hypoglycemic episodes and in microvascular complications in a high percentage of patients [13]. In the year 2000, islet

transplantation was performed using the “Edmonton protocol” that consisted of induction therapy with a monoclonal antibody (mAb) against the interleukin-2 (IL-2) receptor (Daclizumab), and maintenance therapy with a calcineurin inhibitor (CNI, Tacrolimus) and a mammalian target of rapamycin (mTOR) inhibitor (Sirolimus). Over a followup period of approximately 11.9 months, all patients were insulin-free [14]. Subsequently, the reproducibility of this protocol was demonstrated by several studies. For instance, in 2006 an international multicenter trial demonstrated successful restoration of endogenous insulin production and glycemic stability in 44% of transplant recipients at one year following the final islet transplantation. Persistent graft function defined by detectable C-peptide levels and improved glycemic control was observed in 70% of the recipients at the two-year followup although the insulin independence rate was disappointingly low (14%) [15]. The 2008 update from the Collaborative Islet Transplant Registry (CITR) summarizing the results from 279 recipients of one or more islet alone allotransplants between 1999 and 2007 also reported achievement of insulin independence in 23% of islet-alone recipients at 3-year after first infusion (insulin independence ≥ 2 weeks) while 29% were insulin dependent with detectable C-peptide, 26% had lost function, and 22% had missing data [16]. Furthermore, 70% comprising all those with one or multiple infusions achieved insulin independence at least once, of whom 71% were still insulin dependent 1 year later and 52% at 2 years translating to 50% achieving and retaining insulin independence for 1 year and 35% for 2 years for all islet-alone recipients. These results are consistent throughout the 8 years of followup included in the registry. A dramatic decrease in the prevalence of hypoglycemic episodes and substantial improvement in HbA1c levels was also observed. An increase from 2% preinfusion to 47%–69% at year 1 post-last infusion was observed in islet-alone recipients category with HbA1c less than 6.5% and absence of severe hypoglycemic episodes. Factors favoring the positive primary outcomes included amongst others a higher number of infusions, greater number of total islet equivalents infused, lower pre-transplant HbA1c levels, related processing/infusion centers, islet viability more than 87%, larger islet size, and the use of protocols with daclizumab, etanercept or calcineurin inhibitors in the immunosuppressive regimens [16]. In fact, while successful islet transplantation outcomes have also been reported using islets isolated from a single donor [17, 18], Matsumoto et al. were the first to perform successful living donor islet transplantation to a recipient having brittle insulin-dependent diabetes with hypoglycemic unawareness [19]. Results obtained from recipients of autologous islet transplantation after pancreatectomy at the University of Minnesota demonstrated a remarkably limited rate of decline of insulin independence despite infusion of a lower β -cell mass [20]. Of 173 islet autotransplant recipients, insulin independence was achieved in 32% within the first year. Of those with insulin independence, 74% remained insulin independent at the 2-year followup, 46% at 5 years and 28% at 10 years. These results are remarkably higher than the CITR data and highlight the strong impact of

autoimmune and alloimmune injury on graft survival in type 1 diabetics receiving an infusion of allogeneic islets [16, 20, 21]. The Edmonton protocol can also be applied to patients undergoing either islet after kidney (IAK) or simultaneous islet kidney (SIK) procedures resulting in a high rate of graft function and insulin independence, although the morbidity is higher when compared to patients undergoing solitary islet transplantation for brittle T1D [22]. In fact, an improvement in cardiovascular function for up to 3 years of follow-up was observed in type 1 diabetic patients with end-stage renal disease (ESRD) receiving an IAK transplant compared with the kidney-only group. Also improvements in kidney graft function, survival rates, and stabilization of microalbuminuria among uremic patients [23] as well as an improvement in vascular diabetic complications [24] were observed following IAK transplantations. Currently, several Phase I and Phase II clinical trials to study IAK in T1D are ongoing (ClinicalTrials.gov Identifier: NCT00708604; NCT00888628; NCT01123187; NCT00468117). Although restoration of endocrine function and glucose homeostasis can be achieved by transplantation of either whole pancreas or islets of Langerhans, each procedure has distinct advantages over the other. While whole pancreas transplantation has been shown to be very effective at maintaining an euglycemic state over a sustained period of time providing the graft recipient an opportunity to benefit from the improvement of blood glucose control, it is associated with a significant risk of surgical and postoperative complications [25]. Islet transplantation on the other hand, offers substantial benefits in terms of being minimally invasive, reducing incidences of debilitating hypoglycemic episodes/hypoglycemic unawareness, lowering daily insulin requirements, improving levels of glycated hemoglobin, and affording potential insulin independence [26–28]. Furthermore, it allows a window of opportunity for attempting various therapeutic manipulations of islets prior to transplantation aimed at achieving superior transplant outcomes. However, despite the impressive advances in this field, a limiting supply of islets, inadequate means for preventing islet rejection and the deleterious diabetogenic and nephrotoxic side effects associated with chronic immunosuppressive therapy preclude its widespread applicability. Also, long-term insulin independence in islet transplant recipients is frequently lost by the fifth year of followup [29]. Nonetheless, the overall positive impact of islet transplantation on metabolic control in T1D continues to spur efforts worldwide to develop new strategies directed at achieving long-lasting insulin independence following islet transplantation.

2.1. Immune Strategies That Promote Engraftment and Function of Transplanted Islets. Current challenges to successful transplantation include amongst others (a) the loss of functional islet mass resulting from nonspecific inflammatory responses as well as from preexisting and/or transplant-induced, autoimmune-mediated islet destruction and alloimmune rejection and (b) the failure of the newly transplanted islets to revascularize successfully resulting in inefficient engraftment and primary dysfunction of islets.

Herein, we will review some of the various preclinical and clinical efforts to protect islet grafts from the deleterious effects of innate and adaptive allo- and autospecific immune responses as well as to promote vasculogenesis, thereby increasing longitudinal graft survival and function. These strategies include amongst others immunomodulatory therapies and immunosuppressive regimens. Immunomodulatory therapies (e.g., monoclonal antibody therapies, costimulatory signaling blockade, IL-1 receptor antagonist therapy, cellular therapies, etc.) act by (a) providing immunoregulatory cytokines such as IL-4, IL-10, or TGF- β or (b) by inhibiting proinflammatory cytokines or (c) by altering the balance between TH1 and TH2 cells or (d) by protecting islets from immune destruction by encapsulation and so forth. Immunosuppressive regimens on the other hand act by either (a) suppressing the innate and adaptive immune response through various strategies that include binding to specific cytoplasmic proteins that inhibit IL-2 secretion and subsequent T-cell expansion (CNIs Cyclosporine and Tacrolimus) or (b) by suppressing IL-2R signaling thereby inactivating T cells (Sirolimus) or (c) by inhibiting cell division of lymphocytes (Azathioprine) [30]. Currently, combinatorial therapies that promote vasculogenesis/angiogenesis of islets and islet regeneration/ β -cell expansion and that target antigen specific/nonspecific and antibody-specific immunomodulations that readjust the underlying immunologic imbalance to stop/reverse the β -cell-specific immune destruction and maintain immune tolerance together with steroid-sparing or -free immunosuppressive regimens lacking nephrotoxicity or diabetogenicity are of immense therapeutic value towards generating successful islet transplantation outcomes.

2.1.1. Blocking the Instant Blood Mediated Inflammatory Reaction: Pharmacologic and Encapsulation Strategies. One of the first inflammatory assaults encountered by the islets on contact with ABO-compatible blood following transplantation via hepatic portal vein is the instant blood-mediated inflammatory reaction (IBMIR) [31, 32]. IBMIR is a thrombotic reaction characterized by activation of both the coagulation and complement cascades that results in the disruption of islet morphology and function, posing a serious obstacle to successful islet engraftment. Activation of the complement and the generation of anaphylatoxins C3a and C5a leads to recruitment of polymorphonuclear (PMN) leukocytes, upregulation of adhesion molecules on the endothelium and platelets, generation of ROS, and induction of cytokine release [33, 34]. Islets themselves secrete both tissue factors (TFs) that trigger IBMIR [35] and chemokines including monocyte chemoattractant protein 1 (MCP1), chemokine C-X-C motif ligand 8 (CXCL8), and chemokine C-C motif ligand 2 (CCL2) that might participate in recruitment of PMNs and monocytes/macrophages to the site of engraftment [36–39]. In fact, a significant association between TF and CCL2 released *in vitro* by islets and biochemical indices of coagulation was observed in patients after islet transplantation [36] indicating that reduction of the islet proinflammatory state may be a means to reduce the early posttransplant complications and possibly improve islet

engraftment. To this end, strategies attempted for improving β -cell graft survival include the use of OptiPrep gradient media during purification of human islets. This media has been shown to significantly reduce cytokine/chemokine production including IL-1 β , TNF- α , IFN- γ , IL-6, IL-8, MIP-1 β , MCP-1, and RANTES during the 48-hour culture after isolation thereby improving β -cell survival during pretransplant culture [40]. Similarly, embedding mouse islets in reconstituted basement membrane extract (BME) for 24 or 48 hours partially protected islets from anoikis by decreasing caspase-3 levels and increasing α 3 integrin, focal adhesion kinase (FAK) protein level, and FAK activity suggesting a beneficial role in preservation of viability and function of isolated islets. Furthermore, expression of transcription factor pancreatic and duodenal homeobox 1 (PDX-1) was preserved at 48 hours indicating a positive contribution of BME to cell activity [41].

Pioneering work has been done by the group of Nilsson and Korsgren in developing clinically applicable strategies to inhibit the activation of the coagulation and complement systems and the infiltration of leukocytes into islets that characterize the IBMIR. For instance, they demonstrated the effectiveness of low MW-dextran sulphate in inhibiting IBMIR in a nonhuman primate model of pancreatic islet transplantation, providing the basis for a protocol that can be used to abrogate the IBMIR in pig-human clinical islet xenotransplantation [32]. As a result of their studies, a clinical trial to assess the safety and efficacy of LMW-sulfated dextran on posttransplant islet function in people with T1D is ongoing (ClinicalTrials.gov Identifier: NCT00790439). They also developed a biotin/avidin technique to conjugate preformed heparin complexes to the surface of islets that protected against intraportal thrombosis and inhibited the IBMIR without increasing the bleeding risk or inducing acute or chronic toxicity in the islets [35]. On a similar principle, they demonstrated that human aortic endothelial cell- (EC-) coated islets reduced activation of coagulation and complement, platelet/leukocyte consumption in blood as well as infiltration of CD11b+ cells [42, 43]. Furthermore, addition of mesenchymal stem cells (MSCs) to this composite indicated enhanced EC proliferation, sprout formation, and ingrowth of ECs into the islets demonstrating the clinical potential of composite EC-MSC islets in improving islet angiogenesis, revascularization, and immune regulation [44]. Optimizing the EC-islet coculture methods to enable human EC coating of pig islets would offer new strategies to improve xenogenic islet transplantation outcomes. Recently, immobilization of human soluble complement receptor 1 (sCR1) on the islet cell surface through poly(ethylene glycol)-conjugated phospholipid (PEG-lipid) was shown to effectively inhibit complement activation and protect islets against attack by xenoreactive antibodies and complement without loss of islet cell viability or insulin secretion ability, demonstrating an efficient means to control early islet loss in clinical islet transplantation [45]. Coating islet surfaces with PEG-lipid followed by further modification with a fibrinolytic enzyme urokinase further increased the efficacy of this procedure [46, 47]. These reports highlight the potential of tissue-targeted chemistry to reduce donor-cell-

mediated procoagulant and proinflammatory responses. The anaphylatoxin C5a elicits a broad range of proinflammatory effects and most likely plays a crucial role in IBMIR. In a recent mouse intraportal islet transplant study, Satomi's group demonstrated that C5a inhibitory peptide (C5aIP) significantly suppressed the thrombin-antithrombin complex formation, improving both curative rate and glucose tolerance as well as significantly suppressing the expression of TF on granulocytes in recipient livers [48]. Suppression of TF expression attenuated cross-talk between the complement and coagulation cascades leading to improvement in islet engraftment indicating that C5aIP therapy in combination with conventional anticoagulants could represent a beneficial intervention in clinical islet transplantation. Similar reduction in the expression of TF and MCP-1 in human islets was demonstrated by the addition of nicotinamide to the culture medium in an *in vitro* loop model [49]. Surface reengineering of pancreatic islets with recombinant azido-thrombomodulin also resulted in a significant reduction in islet-mediated thrombogenicity [50]. Nilsson's group had also demonstrated that administration of a specific thrombin inhibitor Melagatran significantly reduced IBMIR *in vitro* in a dose-dependent manner [51] as did sCR1/heparin therapy [52, 53] or TP10/immune-suppressive drug treatment following intraportal transplantation of porcine islets to cynomolgus monkeys [54]. Other anticoagulant or complement inhibitors to block IBMIR in preclinical studies include N-acetyl L-cysteine that efficiently inhibited the procoagulant activity of human recombinant TF in human islet cell preparations at clinically relevant concentrations without cellular toxicity [55].

Fetal and neonatal porcine islets express a cell surface antigen containing the epitope—Gal α 1—3Gal β 1—4GlcNAc-R (“ α -gal”) to which humans have complement fixing antibodies that cause immediate rejection of transplanted islets [56–59]. This response may be prevented by (a) inhibition of the functional “ α -gal” activity using knockout [60] or knockin procedures involving human alpha (1,2)-fucosyltransferase [61] or N-acetyl-glucosaminyl transferase III gene expression [62] or (b) by depletion of anti-pig antibodies or complement from serum or (c) the use of GT knockout (α 1,3-galactosyltransferase gene knockout [GTKO]) pigs [63] as the sources of islets. Expression of thrombomodulin [64], TF pathway inhibitor [65], CD39 the endothelial ecto-nucleoside triphosphate diphosphohydrolase, and haem oxygenase also demonstrate potential value for coagulation control in pig-to-human xenotransplantation. Prevention of coagulation dysfunction [66] or expression of human complement-regulatory proteins such as CD46 (membrane cofactor protein) [67, 68], human decay accelerating factor (DAF, CD55) [64, 69], CD59 [70–72] or all three [73]; or expression of anticoagulant and antiplatelet molecules within the graft may also afford some protection [57]. For instance, adenovirus-mediated expression of the human complement regulatory proteins DAF (CD55) or CD59 protected adult porcine islets from complement-mediated cell lysis by human serum [74] as did a low-molecular mass factor VIIa (FVIIa) inhibitor that indirectly blocked both membrane bound and alternatively

spliced forms of TF *in vitro* [75]. Recombinant antithrombin III may also ameliorate both early graft damage and the development of systemic coagulation disorders in pig-to-human xenotransplantation [76]. These strategies in parallel with physical methods such as encapsulation may contribute significantly in reducing the thrombogenicity of pig islet xenografts.

Several intravascular and extravascular devices involving encapsulation technology to overcome destruction of the graft by immune cells and large antibodies have been described. While intravascular macrocapsules are anastomosed to the vascular system as AV shunt, extravascular macrocapsules are mostly diffusion chambers transplanted at different sites and extravascular microcapsules are transplanted in the peritoneal cavity. The major advantage of the intravascular device is that direct contact with the blood stream ensures ample oxygen and nutrient supply enhancing graft survival and function. Use of vascularized bioartificial devices was among the few cases that obtained long-term allo- and xenogeneic islet survival in totally pancreatectomized dogs [77, 78]. However, thrombosis is a major challenge, requiring intense anticoagulation therapy. The extravascular devices on the other hand offer minimal surgical risk, transplantation at extrahepatic sites, lack of thrombosis, and ease of retrieval from recipient in case of pericapsular fibrotic overgrowth. Both these devices however remain vulnerable to small molecules such as cytokines/chemokines and nitric oxide as well as hypoxic stress. Coencapsulation of islets with various agents such as erythrocytes [79] and sertoli cells [80] that release immunosuppressive factors or with factors that enhance revascularization such as vascular endothelial growth factor (VEGF) [81], photosynthetic oxygen generator algae [82] and so forth or with factors like bioengineered insulin-like growth factor-II (IGF-II) [83] that promote pancreatic β -cell survival have been shown to improve insulin secretion and glycemic control posttransplantation. Naturally obtained alginate hydrogels are most widely used in islet transplantation with barium alginate demonstrating long-term immunoprotection in both allo- and xenotransplantation settings. For instance, a significant decrease in the secretion of MCP1 and improvement in the islet activity was observed when SD rat islets were microencapsulated with novel sulfated glucomannan barium alginate (SGA) and allotransplanted intraperitoneally into diabetic Lewis rats [84]. Similar immunoprotection was observed when islets co-encapsulated with angiogenic protein in permselective multilayer alginate-poly-L-ornithine-alginate microcapsules were transplanted into an omentum pouch [85]. An extravascular (subcutaneous) transplant macrochamber called the " β Air" device, consisting of islets immobilized in a thin alginate-impregnated, hydrophilized Teflon membrane and simultaneously supplied with oxygen by daily refueling with oxygen-CO₂ mixture provided immunoprotection and sustained islet viability and function following allogeneic subcutaneous transplantation into healthy minipigs [86]. Transplantation of porcine islets microencapsulated in highly purified barium alginate into streptozotocin- (STZ-) induced diabetic Wistar rats resulted in long-term normoglycemia

without immunosuppression [87]. Thus, by providing a solution to the hurdles of immunological rejection and the risk of infection with porcine pathogens in clinical xenoislet transplantation [88], the use of encapsulation devices may help to circumvent the shortage of allogeneic human donor organs [56]. Alginate macroencapsulation of pig islets was also shown to correct STZ-induced diabetes in primates up to 6 months without immunosuppression [89]. In a pilot clinical trial wherein microencapsulated human islets were transplanted into the central abdominal region of non-immunosuppressed patients with T1D, improvement in glycated hemoglobin and the disappearance of hypoglycemia was observed, although the patients remained on insulin therapy [90]. In a very recent clinical study, Elliot et al. demonstrated transitory insulin independence of several months duration using the microencapsulation technique [56]. Interestingly, the treatment appeared to significantly decrease severe hypoglycemic episodes and reduce/abolish hypoglycemic unawareness episodes, even in the absence of insulin independence. Evidence of xenosis in the xenotransplants recipients though diligently sought could not be found.

2.1.2. Immunotherapeutic Strategies Targeting the Non-Antigen Specific Immune Response. Approximately 60% of transplanted pancreatic islet tissue undergoes apoptosis within the first several days in experimental models of syngeneic islet transplantation [13, 91, 92]. Infiltration of graft by PMNs and tissue macrophages and elevated levels of proinflammatory cytokines following islet transplantation strongly suggest the involvement of nonspecific innate inflammatory events in mediating cellular injury. Other mediators involved in the early loss of pancreatic islets include NO, prostaglandins (PGs), and reactive oxygen intermediates (ROIs) [92, 93]. Several studies indicate that inhibition of non specific inflammation improves the function and survival of islet grafts. For instance, Yasunami's group demonstrated that V-alpha14 NKT cell-triggered IFN- γ production by Gr-1+CD11b+ cells mediated early graft loss of syngeneic transplanted islets [94]. Adenosine administration suppressed NKT cell-mediated IFN- γ production of neutrophils in the livers of graft-recipient mice, leading to prevention of early loss of transplanted syngeneic and allogeneic islets. Similar results were observed in a recent study wherein recipients receiving ATL therapy (ATL146e or ATL313) achieved normoglycemia more rapidly than untreated recipients following syngeneic islet transplantation indicating improved survival and functional engraftment of transplanted marginal mass of islets [91]. Histological examination of grafts suggested reduced cellular necrosis, fibrosis, and lymphocyte infiltration in agonist-treated animals. Administration of adenosine A(2A) receptor agonists also improved *in vitro* glucose-stimulated insulin secretion (GSIS) by an effect on leukocytes, suggesting a potentially significant interventional strategy for reducing inflammatory islet loss in clinical transplantation. A similar beneficial effect associated with reduction in inflammatory cell infiltration and β -cell death by apoptosis was observed following

administration of diannexin [95]. Sequential combination of a JNK inhibitor SP600125 and nicotinamide plus simvastatin also protected porcine islets from peritransplant apoptosis and inflammation [96]. This combination therapy increased β -cell viability index and viability of porcine islets cultured overnight as well as significantly increased the islet survival rate *in vivo*. Intraductal administration of JNK inhibitor significantly suppressed mRNA expression levels of IL-1 β , IFN- γ , TNF- α , IL-6, IL-8, and MCP-1 *in vivo* and also decreased the concentration of IL-1 β and IL-8 in culture supernatant *in vitro* possibly representing an alternative target for suppression of porcine islet inflammation. Very recently, administration of bilirubin too was shown to reduce the serum levels of inflammatory mediators including IL-1 β , TNF- α , soluble intercellular adhesion molecule 1 (ICAM1), MCP-1 and NO, inhibit the infiltration of Kupffer cells into islet grafts, restore insulin-producing ability of transplanted islets and enhance glucose tolerance in diabetic mouse recipients [97]. IL-1 β plays a key role in causing pancreatic islet dysfunction and apoptosis [92]. Through a cascade of intracellular events, IL-1 β secreted by neutrophils and macrophages was shown to down-regulate GLUT2, up-regulate inducible NO synthase (iNOS) and cyclooxygenase-2, and activate NF- κ B which in turn mediated the transcription of a multitude of genes, including IL-1, IL-6, TNF- α , ICAM-1, VCAM-1, ELAM-1, iNOS, PGE2, COX-2, and EP3 [92, 97–99]. The inhibition of IL-1 β induced COX-2 and EP3 gene expression by sodium salicylate [99] as well as of iNOS and COX-2 tyrosine kinase were all shown to enhance pancreatic islet β -cell function [100]. Also deficiency of NOX2 decreased ROI/proinflammatory cytokine production and β -cell apoptosis, thereby protecting against STZ-induced β -cell destruction and development of diabetes in mice [101].

Inhibition of Toll-Like Receptor (TLR) Signaling. A family of 10 functional, human, innate, immune signaling receptors called TLRs plays an important role in the host's innate defense as well as adaptive immunity. Recognition of conserved pathogen-associated microbial patterns (PRRs) stimulates specific TLRs on APCs and T lymphocytes to induce proinflammatory cytokines, chemokines, and interferons. The endogenous TLR ligands include amongst others peptidoglycan, extracellular domain A of fibronectin, lipopolysaccharide, heparan sulfate, hyaluronan, high-mobility group box protein 1 (HMGB1), Hsp60, exogenous products such as bacterial lipoprotein, CpG DNA, flagellin, and double stranded viral RNA [102–105]. Murine islets constitutively express TLR2 and TLR4, and TLR activation upregulates intra-islet production of cytokines and chemokines. A recent study indicated that TLR2 and TLR4 signaling could initiate islet graft failure and that HMGB1 was an early mediator [106]. Following transplantation into STZ-induced diabetic syngeneic mice, islets exposed to LPS or peptidoglycan had primary graft failure caused by recipient CD8+ T cells with intra- and peri-islet mononuclear cell inflammation. NF κ B activation in stressed islets was prevented in the absence of both TLR2 and TLR4. Transplantation of TLR2/4(-/-) islets reduced proinflammatory cytokine production and

improved islet survival. Yet another study indicated that early intraportal islet graft failure in mice was associated with increased proinflammatory cytokines, HMGB1 expression, NF κ B activation, caspase-3, and TUNEL-positive cells [107]. Deficiency of TLR4 in donor, but not in recipient, inhibited NF κ B activation, reduced proinflammatory cytokines and improved viability of islet grafts. Blockade of HMGB1 with anti-HMGB1 monoclonal antibody (mAb, 2 g7) inhibited inflammatory reactions as evidenced by reduced TNF α and IL-1ss production and improved islet viability. Thus, these results indicate that inhibition of TLR4 activation represents a novel strategy to attenuate early graft failure following intraportal islet transplantation. Matsuoka et al. demonstrated that mice HMGB1 receptors TLR2 or receptor for advanced glycation end products (RAGE) but not TLR4, failed to exhibit early islet graft loss [108]. Mechanistically, HMGB1 stimulated hepatic mononuclear cells (MNCs) *in vivo* and *in vitro*, upregulated CD40 expression and enhanced IL-12 production by DCs, leading to NKT cell activation and subsequent NKT cell-dependent augmented IFN- γ production by Gr-1+CD11b+ cells. Treatment with either IL12- or CD40L-specific antibodies prevented early islet graft loss. Also, treatment with a HMGB1-specific antibody inhibited IFN- γ production by NKT cells and Gr-1+CD11b+ cells following intraportal islet transplantation, indicating that the HMGB1-mediated pathway was a potential interventional target for improving the efficiency of islet transplantation.

Protection by Suppressor of Cytokine Signaling (SOCS). The protective effect of suppressor of cytokine signalling (SOCS)-3 in mouse and rat islets subjected to cytokine stimulation has been characterized by Rønn et al. [109]. Using mice with β -cell-specific Socs3 expression as well as a Socs3-encoding adenovirus construct, a significant resistance to cytokine-induced apoptosis and impaired insulin release was demonstrated by the transgenic islets. GSIS, insulin content or glucose oxidation were not affected by SOCS3. Rat islet cultures transduced with Socs3-adenovirus too displayed reduced cytokine-induced NO and apoptosis associated with inhibition of the IL1-induced NF κ B and mitogen-activated protein kinase (MAPK) pathways. While transplanted Socs3 transgenic islets were not protected in diabetic NOD mice, they did show a prolonged graft survival when transplanted into diabetic allogeneic BALB/c mice indicating that SOCS3 may represent a target for pharmacological or genetic engineering in islet transplantation for treatment of T1D. A more recent study using chimeric adenovirus vector (Ad5F35-SOCS1) to enhance SOCS1 expression in isolated Sprague-Dawley rat islets indicated decreased levels of active caspase 3 and intranuclear apoptosis inducing factor (AIF) after treatment with TNF- α and cyclohexamide *in vitro* [110]. Caspase 3 is the central executioner caspase activated by upstream cascades in a caspase-dependent apoptosis pathway while AIF is a key mitochondrial protein that translocates to the nucleus in a caspase-independent apoptosis pathway. Transplantation of Ad5F35-SOCS1-infected islets into STZ-induced diabetic recipients resulted in significantly prolonged functional graft survival. Also, decreased caspase 3

activation and AIF translocation to nucleus was observed in Ad5F35-SOCS1-infected islet grafts in the early posttransplant period indicating that SOCS1 mediated protection of islet grafts from apoptosis through caspase-3-dependent and AIF-caspase-independent pathways [110]. These results were supported by another study in which transgene expression of SOCS-1 rendered islets significantly more resistant to cytokine-induced cell death after treatment with TNF- α alone and in combination with IFN- γ [111]. Furthermore this resistance could be correlated with significant inhibition of the transcription factor interferon regulatory factor-1 (IRF-1), reflecting enhanced cell survival signals as well as impairment of IFN γ -induced class I MHC upregulation [93, 111]. Importantly, SOCS1-Tg islets significantly reversed STZ-induced diabetes indicating that intragraft expression of SOCS1 rendered islets insensitive to the deleterious effects of cytokines; a finding of potential significance in the development of therapies against acute allograft rejection [111].

Protection by X-linked Inhibitor of Apoptosis Proteins (XIAPs). Adenovirally delivered, transient overexpression of X-linked inhibitor of apoptosis proteins (XIAPs) in a growth-regulatable β -cell line (β TC-Tet) indicated that *in vitro*, XIAP-expressing β TC-Tet cells were markedly resistant to apoptosis in an ischemia-reperfusion injury model system as well as following exposure to cytokines [112]. Subcutaneous transplantation of these Ad-XIAP transduced β TC-Tet cells into immunodeficient mice resulted in reversal of diabetes in 3 days as versus 21 days for Ad- β Gal transduced control cells. These results were recapitulated in a recent study wherein XIAP overexpression inhibited β -cell apoptosis in syngeneic islet transplants, reducing the number of islets as well as decreasing the number of days required to restore euglycemia, thereby raising the possibility that *ex vivo* XIAP gene transfer in islets prior to transplantation had the potential to increase donor islet mass available for transplantation by allowing more efficient use of the limited existing supply of human islets thereby enhancing graft function and long-term transplant success [113]. In comparison with this adenoviral delivered gene transfer approach, a “nonviral” approach was recently described involving the transfection of IDO cDNA-containing plasmids into rat islets using Lipofectamine. Indoleamine 2,3-dioxygenase (IDO) is an enzyme that plays a critical role in suppressing T-cell responses inducing fetomaternal tolerance [114]. Allograft transplantation of IDO expressing islets into STZ-induced diabetic Lewis rats was shown to reverse diabetes and maintain glucose metabolic control. Also, survival of IDO-transfected islet allografts transplanted without any immunosuppression was superior to that observed in diabetic rats receiving nontransfected islets.

Protective Effect of Haem Oxygenase-1 (Ho-1, Hmox1). Overexpression of the cytoprotective protein haem oxygenase-1 (HO-1) has been shown to reduce the deleterious effects of cytokine-induced apoptosis and oxidative stress in transplantable islets [115–118]. For instance, HO-1 upregulation

by protoporphyrins (Cobalt-protoporphyrin CoPP, Ferrous-protoporphyrin FePP), powerful inducers of the HO-1, in a murine insulinoma β -TC3 cell line and in freshly isolated murine islets was shown to exert a protective effect from apoptosis induced *in vitro* with proinflammatory cytokines [115, 116] or Fas engagement [117], while *in vivo* HO-1 upregulation resulted in improved islet function in a syngeneic model of marginal mass islet transplantation in rodents [118]. These results were supported by a similar study in which CoPP treatment of donor mice for the induction of HO-1 beginning one-day prior to islet isolation plus a 9-day posttransplantation course resulted in enhanced engraftment of syngeneic islets and improved blood glucose levels and glycemic control [119]. Short-course peritransplant administration of CoPP also led to long-term DBA/2 islet allograft survival in a sizable proportion of C57BL/6 mice recipients [120]. Furthermore, preconditioning of islets with FePP alone led to improved graft survival in untreated recipients and further increased the proportion of long-term surviving grafts in CoPP-treated recipients. Preconditioning also resulted in reduction of class II expression. Peritransplant administration of protoporphyrins to allograft recipients also resulted in transient powerful immunosuppression with reduced lymphocyte proliferative responses, increased proportion of regulatory T cells (CD4+CD25+) and decreased mononuclear cell infiltrating the graft, paralleled by a systemic upregulation of HO-1 expression, probably contributing to the induction of donor-specific hyporesponsiveness in a proportion of the protoporphyrin-treated animals. The transgenic expression of haem oxygenase-1 in pancreatic β -cells of NOD mice prolonged graft survival and afforded protection from autoimmune damage [121]. Reduced levels of proinflammatory cytokines/chemokines, proapoptotic gene expression, and amounts of ROS/RNS from islets were observed, with islets more resistant to TNF α - and IFN γ -induced apoptosis, providing valuable insight into the development of better strategies for clinical islet transplantation in patients with T1D.

Protective Regulatory Role of Interferon Regulatory Factor 1 (IRF1). A key role of IRF1 in immune-mediated β -cell destruction has been indicated [93]. IRF-1 is a downstream target of IFN- β /signal transducer and an activator of STAT-1. Deletion of IRF1 in islets was associated with a higher prevalence of primary nonfunction, reduced insulin secretion and shorter functioning graft survival. Cytokine-exposed Irf1 ($-/-$) islets and INS1E cells transfected with Irf1 siRNA showed increased expression of Mcp1 (Ccl2), Ip10 (Cxcl10), Mip3 α (Ccl20), and Inos (Nos2) mRNA and elevated production of MCP-1 and nitrite compared with controls. *In vivo*, Irf1 ($-/-$) islets displayed a higher potential to attract immune cells, reflected by more aggressive immune infiltration in the grafted islets. IL-1 receptor antagonist partly restored the cytokine-induced secretory defect *in vitro* and completely prevented primary non function *in vivo*. These data indicate a key regulatory role for IRF1 in insulin and chemokine secretion by pancreatic islets under inflammatory attack.

Beneficial Effect of Redox Modulation Strategies. β -cells are especially vulnerable to free radical and inflammatory damage due to reduced antioxidant defenses. A recent study was done to determine the efficacy and benefit of a redox modulation strategy using the catalytic antioxidant (CA) FBC-007 in improving islet preservation. The results indicated that incubation of human islets with FBC-007 before syngeneic, suboptimal syngeneic, or xenogeneic transplant protected islets from STZ-induced damage and significantly increased their function [122]. Diabetic murine recipients of catalytic antioxidant-treated allogeneic islets exhibited improved glycemic control posttransplant and demonstrated a delay in allograft rejection. Systemic administration of catalytic antioxidant to recipients further delayed allograft rejection suggesting that addition of a redox modulation strategy would be a beneficial clinical approach for islet preservation in syngeneic, allogeneic, and xenogeneic transplantation.

2.1.3. Targeting Adaptive Immunity: The Effectiveness of Immunosuppressive and Tolerance-Inducing Immunotherapeutic Strategies. Presentation of alloantigens in combination with costimulatory molecules by APCs activates signal transduction pathways, including the calcium-calcineurin pathway, triggering the T-cell response [21]. Subsequent release of IL-2 amongst other molecules, activates the mTOR pathway initiating cell proliferation and hugely expanding the number of effector T cells. Activated B lymphocytes too produce alloantibodies against donor HLA antigens.

Costimulatory Signaling Blockade. Several studies indicate that blockade of costimulatory signal pathways in experimental transplantation models by cytotoxic T lymphocyte antigen-4 immunoglobulin (CTLA4Ig) or CD40Lig enhances islet graft survival [123, 124]. For instance, human islets infected with AdCTLA4Ig-IRES2-CD40Lig that simultaneously expressed CTLA4Ig and CD40Lig proteins significantly prolonged graft survival of murine islet xenografts, downregulated expressions of Th1-cells-related cytokines, and inhibited inflammatory cell infiltration [125]. Caspase inhibitor therapy (EP1013) in combination with costimulation blockade (CTLA4-Ig) too prevented engraftment phase islet loss and markedly reduced islet mass required to reverse diabetes [126]. Furthermore, EP1013/CTLA4-Ig cotherapy significantly increased graft survival by reducing the frequency of alloreactive IFN- γ secreting T cells and increasing the frequency of intragraft FoxP3+ Treg cells. The results of these studies indicate that by minimizing immune stimulation and reducing the requirement for long-term immunosuppressive therapy, these combination therapies have tremendous potential in improving clinical transplantation.

Simultaneous blockade of CD40/CD154 and ICAM/lymphocyte function-associated antigen (LFA)-1 also prolonged allograft survival [127]. Larsen's group targeted adhesion molecule LFA-1 that is preferentially expressed on donor-specific memory T (TM) cells and has been impli-

cated in costimulation blockade-resistant transplant rejection [128]. Short-term induction therapy with the LFA-1-specific antibody TS-1/22 in combination with either Basiliximab (an IL-2R α -specific mAb) and Sirolimus or Belatacept (a high-affinity variant of the CD28 costimulation-blocker CTLA4Ig) prolonged islet allograft survival in nonhuman primates by masking LFA-1 on TM cells. Inhibition of the generation of alloproliferative and cytokine-producing effector T cells expressing high levels of LFA-1 *in vitro* was also observed. Neutralization of costimulation blockade-resistant populations of T cells with LFA-1-specific induction therapy has tremendous potential in transplantation. The efficacy of Efalizumab, a blocking monoclonal antibody directed at LFA1, as part of maintenance therapy regimen with Sirolimus was being tested (ClinicalTrials.gov identifier NCT00672204). Efalizumab is no longer available for clinical use. An anti-coinhibitory receptor B and T lymphocyte attenuator monoclonal antibody (anti BTLA, PJ196) has been reported to prolong fully MHC-mismatched cardiac allograft survival [129]. A study testing the synergistic effect of anti-BTLA monoclonal antibody PJ196 and CTLA4Ig costimulatory blockade in islet allotransplantation showed that downregulation of BTLA on the surface of lymphocytes along with accumulation of cells with regulatory phenotype at the graft site promoted islet allograft acceptance indicating that this combination may prove to be an effective adjunctive strategy for inducing long-term allograft survival. Similarly, co-stimulatory signal blocker LEA29Y (Belatacept) has shown promising data in transplantation studies in primates [130]. Clinical trials using LEA29Y in islet transplantation are currently underway, for example Clinical Islet Transplantation consortium's clinical trial CIT-04 is designed to study islet transplantation in type 1 diabetes with LEA29Y (belatacept) maintenance therapy (University of Alberta, Emory University) and another ongoing Phase II interventional study (ClinicalTrials.gov Identifier: NCT00501709) is using Belatacept for the prevention of autoimmune destruction and rejection of human pancreatic islets following transplantation for T1D. Apart from costimulation blockade of the CD28/CD80/CD86 and CD40/CD154 pathways as a means of inducing peripheral tolerance, several new costimulatory molecules have been identified in recent years including OX40 that belongs to the TNF receptor family and is expressed on activated T cells [131]. OX40 ligation with OX40L enhances cytokine production, proliferation, and survival [131]. Mechanistic studies indicate that anti-OX40L, treatment preserves Treg numbers and OX40 blockade offers better xenoislet graft survival than CTLA4Ig in the spontaneous autoimmune NOD model, offering a novel therapeutic target for xenoislet graft protection in type 1 diabetic patients [132]. Recently, a combination therapy consisting of anti-CD40L, anti-OX40L, and anti-CD122 mAbs prolonged islet allograft survival in alloantigen-primed mice [133]. This combination of mAbs also inhibited accelerated rejection mediated by donor reactive memory T cells, known to accelerate allograft rejection, in xenoantigen-primed mice cells by inhibiting cellular and humoral immune responses.

Monoclonal Antibodies. Anti-CD3 antibody has been shown to induce tolerance in allograft transplantation [134], reverse autoimmunity in NOD mice [135] and slow the progression to permanent diabetes in humans with recent-onset diabetes [136]. In female NOD mice, oral anti-CD3 mAb was effective in reversing diabetes, allowing pregnancies and extending longevity [135]. Treatment of diabetic transgenic mice (NOD background) expressing the human ϵ chain of the CD3 complex with Otelixizumab (an anti-human CD3 antibody) resulted in durable disease remission dependent on transferable T-cell-mediated tolerance [137]. In single-donor islet transplantation studies [138], anti-CD3 mAb [hOKT3c1(Ala-Ala)] contributed to promising results [139]. A Phase II clinical trial studying the efficacy of anti-CD3 mAb in the treatment of recent onset T1D (ClinicalTrials.gov identifier NCT00378508) is currently underway. Another trial studying the efficacy of hOKT3 γ 1 (Ala-Ala) combined with Sirolimus and delayed Tacrolimus in promoting islet allograft survival has just been completed. In an adoptive transfer model, the use of anti-CD134L mAb was shown to effectively prevent activation of CD4+ memory T cells and significantly prolong islet survival, similar to the manner anti-CD122 mAb prevents activation of CD8+ memory T cells [140]. Short-term administrations of a combination of anti-LFA-1 and anti-CD154 monoclonal antibodies too induced tolerance to neonatal porcine islet xenografts in mice [141]. The use of anti-CD134L and anti-CD122 mAbs in addition to co-stimulatory blockade with anti-CD154 and anti-LFA-1 prolonged secondary allograft survival in an alloantigen-primed model and significantly reduced the proportion of memory T cells [142]. It also increased the proportion of Tregs in the spleen, inhibited lymphocyte infiltration in the graft, and suppressed alloresponse of recipient splenic T cells suggesting that the combination therapy of four mAbs could significantly suppress the function of memory T cells and prolong allograft survival in alloantigen primed animals. Similar results were obtained by the same group using a combination of CTLA4Ig, antiCD40L, anti-LFA-1, and anti-OX40L while another study used a combination of anti-CD40L, anti-OX40L, and anti-CD122 mAbs antibodies to inhibit accelerated rejection mediated by memory T cells in xenoantigen-primed mice [143].

IL-21-Targeted Therapy. The efficacy of an IL-21-targeted therapy (IL-21R/Fc, an IL-21-neutralizing chimeric protein) on prevention of diabetes in NOD mice, in combination with syngeneic islet transplantation has recently been investigated [144]. Results showed that IL-21-responsiveness by CD8+ T cells was sufficient to mediate islet allograft rejection and that combining neutralization of IL-21 with islet transplantation restored glucose homeostasis and resulted in recovery from autoimmune diabetes. Since the absence of IL-21 signaling prevented islet allograft rejection indicating a robust influence of IL-21 on a graft-mounted immune response, these findings imply that therapeutic manipulation of IL-21 may serve as a suitable treatment for patients with T1D.

DNA Vaccination Strategy. A therapeutic DNA vaccination strategy for autoimmunity and transplantation has also

recently been described wherein intradermal injection of plasmid DNA encoding glutamic acid decarboxylase (GAD) polypeptide, which is synthesized in both pancreatic islet and skin tissue, ameliorated new-onset T1D in NOD mice and increased skin allograft survival in a BALB/c-C57BL/6 model system in a donor-specific manner [145]. Furthermore, only CpG-methylation of plasmid DNA coding for GAD was required in order to significantly increase skin allograft survival after immunization of recipient; codelivery of a cDNA coding for the proapoptotic BAX protein, which was shown previously to be essential for successful therapy of autoimmune diabetes in NOD mice by induction of FoxP3+ regulatory T cells, was not necessary. This study therefore revealed the promising potential for autoimmunity-targeting DNA vaccination to be applied to clinical transplantation.

Localized Immunosuppression Using Glucocorticoid Microspheres. The ability of poly(D,L-lactic) acid (PLA) and poly(D,L-lactic glycolic acid (PLGA) microspheres of the soft corticosteroid loteprednol etabonate (LE) loaded within a biohybrid device to provide localized immunosuppression and reduce systemic side effects over an extended period has been evaluated in a set of early exploratory experiments with diabetic rats receiving islet transplantation. While sustained release of microspheres at low concentration showed no cytotoxicity on the viability of the MIN-6 insuloma cell line *in vitro*, animals treated using a biohybrid device loaded with microspheres showed improved results compared to those treated by delivery in solution form with an osmotic minipump [146]. Sustained local delivery formulation for glucocorticoids dexamethasone phosphate and LE using PLA microspheres showed promise in their ability *in vivo* to prolong allograft survival in rats after tapering of systemic immunosuppression, compared to control groups [147]. Also, doses delivered locally were approximately hundredfold smaller than those typically used in systemic treatments.

Induction of Tolerance by Regulatory T cells (Tregs). Donor alloantigen-specific CD4+CD25+ Tregs play an important role in inducing and maintaining tolerance to donor alloantigens *in vivo*. By actively modulating or suppressing the deleterious CD4+ and CD8+ T-cells-mediated immune response to donor alloantigens, Tregs may prevent rejection and mediate linked unresponsiveness [148]. *In vivo*, these cells are dependent on interleukin IL-10 and CTLA4 for functional activity. As part of clinical tolerance strategies, the immunosuppressive ability of peripheral T-cell-depleting agents such as anti-CD52 mAb Alemtuzumab (Campath-1H) and antithymocyte globulin (ATG) have been tested in humans with autoimmunity and in transplantation scenarios [139, 149]. Lopez et al. were the first to report that ATG but not Alemtuzumab or the IL-2R antagonists caused rapid and sustained expansion of CD4+CD25+ T cells in cocultures with human peripheral blood lymphocytes and displayed enhanced expression of the regulatory markers glucocorticoid-induced TNF receptor, CTLA4 and FoxP3 as well as efficient suppression of a direct alloimmune response of the original responder lymphocytes [150]. The induction

of Tregs depended on the production of Th2 cytokines in the generating cultures. This study demonstrated the therapeutic potential of ATG in promoting the generation of Tregs for cellular therapy in autoimmunity and clinical transplantation. Recently, a clinically relevant immunoregulatory strategy based on treatment of NOD mice with murine Thymoglobulin (mATG) and CTLA4-Ig to prevent allo- and autoimmune activation in a stringent model of islet transplantation and diabetes reversal was investigated. The results revealed that transplant recipients experienced a complete abrogation of autoimmune responses and significant downregulation of alloimmunity in response to treatment [151]. Furthermore, this striking effect was confirmed by 100% diabetes reversal in newly hyperglycemic NOD mice and 100% indefinite graft survival of syngeneic islet transplantation (NOD*scid* into NOD mice). An induction immunosuppressive therapy regimen consisting of rabbit ATG and the monoclonal antibody to CD20 rituximab (Rituxan) promoted long-term islet allograft survival in cynomolgus macaques maintained on rapamycin monotherapy [152]. Several clinical trials aimed at studying the safety and effectiveness of islet transplantation combined with various immunosuppressive regimens that include ATG for treatment of type 1 diabetic individuals experiencing hypoglycemia unawareness and severe hypoglycemic episodes are either completed or underway. These include amongst others an islet after kidney transplantation trial with ATG and etanercept (ClinicalTrials.gov Identifier: NCT00468117) and islet transplantation trials with ATG, Everolimus and cyclosporine (ClinicalTrials.gov Identifier: NCT00286624); with Rituxan (Rituximab), Thymoglobulin (ATG), Zenapax (Daclizumab) and Rapamune (Sirolimus) (ClinicalTrials.gov Identifier: NCT00468442); with Lisofylline, ATG, basiliximab, Sirolimus and Tacrolimus, (ClinicalTrials.gov Identifier: NCT00464555); and with Raptiva, ATG, and sirolimus (ClinicalTrials.gov Identifier: NCT00672204) amongst others. In a recent study, patients receiving intraportal allogeneic islet transplants were maintained on immunosuppression consisting of ATG induction and maintenance with sirolimus or mycophenolate mofetil (MMF) and costimulation blocker belatacept or the anti-LFA-1 antibody Efalizumab which permit long-term islet allograft survival [153]. The results indicated that both regimens were effective and well tolerated, with the calcineurin inhibitor/steroid-sparing islet protocols resulting in long-term insulin independence and belatacept proving to be an effective alternative in improving graft function and longevity while minimizing renal and β -cell toxicity. Because Tregs strongly suppress the immune response in syngeneic islet transplantation and improve graft survival and function, several approaches are now emerging to induce/increase host Tregs activity in the transplant setting, including amongst others, systemic TGF- β 1 therapy [154]. A recent study demonstrated that islet-specific Treg induced from the BDC-6.9 TCR transgenic mouse by activation of T cells in the presence of TGF- β could suppress both spontaneous diabetes as well as transfer of diabetes into NOD*scid* mice by diabetic NOD spleen cells or activated BDC-2.5 TCR transgenic Th1 effector T cells [155]. In the latter transfer model, the authors demonstrated that

infiltration of Tregs into pancreas caused a reduction in the number of effector Th1 T cells and macrophages, and also inhibited effector T-cell cytokine and chemokine production. Transfection of effector T cells with a dominant-negative TGF- β receptor demonstrated that *in vivo* suppression of diabetes by TGF- β -induced Treg is TGF- β dependent.

Rapamycin. Rapamycin is a key component of the immunosuppressive regimen in clinical islet transplantation. The impact of rapamycin, on human islet engraftment and function was assessed in 10 patients with T1D before islet transplantation [156]. The results indicated that pretreatment with rapamycin was associated with a reduction in chemokines CCL2 and CCL3 pretransplantation and a dampened chemokine response post-transplantation, potentially improving clinical islet engraftment by an anti-inflammatory mechanism. In rat-to-mouse islet xenotransplantation, administration of anti-CD154 mAb and rapamycin induced Treg-mediated tolerance [157]. Rapamycin has been shown to allow expansion, proliferation, and regulatory function of both murine and human naturally occurring CD4+CD25+FOXP3+ Tregs (nTregs), which are pivotal for the induction and maintenance of peripheral tolerance [158]. Pothoven et al. demonstrated that rapamycin-conditioned Balb/c donor bone-marrow-derived DCs (BMDCs) had significantly enhanced ability to induce CD4+CD25+FOXP3+ iTregs of recipient origin (C57BL/6 (B6)) *in vitro* under Treg driving conditions compared to unmodified BMDCs [154]. These *in vitro* induced CD4+CD25+FOXP3+ iTregs exerted donor-specific suppression *in vitro* and prolonged allogeneic islet graft survival *in vivo* in RAG(-/-) hosts upon coadoptive transfer with T-effector cells. The CD4+CD25+FOXP3+ iTregs also expanded and preferentially maintained FOXP3 expression in the graft draining lymph nodes and were able to induce endogenous naive T cells to convert to CD4+CD25+FOXP3+ T cells. Thus, rapamycin-conditioned donor BMDCs can be exploited for *in vitro* differentiation into donor antigen-specific CD4+CD25+FOXP3+ iTregs capable of effectively controlling allogeneic islet graft rejection. Strom's group demonstrated a triple therapy approach that combined administration of rapamycin and agonist IL-2- and antagonist IL-15-related cytolytic fusion proteins [159]. This treatment promoted very long-term engraftment/tolerance of allogeneic islets in both spontaneously diabetic NOD mice and IL-2-deficient recipients by limiting the early expansion of activated T cells, preserving and even exaggerating their subsequent apoptotic clearance, and further amplifying the depletion of these activated T cells by antibody-dependent mechanisms, while preserving CD4+CD25+ T-cell-dependent immunoregulatory networks. In T1D, Treg activity has been demonstrated in the pancreatic lymph node, but the *in vivo* activity of Tregs during suppression in pancreas remains poorly characterized. A clinical study wherein nTreg numbers and function were examined in a unique set of patients with T1D who underwent rapamycin monotherapy before islet transplantation indicated that while rapamycin monotherapy did not alter the frequency and functional features, namely, proliferation, and cytokine

production of circulating nTregs, it increased their capacity to suppress proliferation of CD4+CD25⁻ effector T-cells [160]. These findings demonstrate that rapamycin directly affects human nTreg function *in vivo*, by refitting their suppressive activity, whereas it does not directly change effector T-cell function.

Induction of Tolerance Through Suppression of TLRs. TLR2-induced IL-6 secretion from APCs has been shown to reverse the suppressive function of Tregs [104, 161] and in combination with TGF β -induced Th17 cells resulted in enhanced inflammation [103, 104, 162, 163] and prevention of transplantation tolerance [163]. In a very recent study to understand the mechanism by which different inflammatory signals affect transplantation tolerance and immunity, it was determined that TLR2 ligand peptidoglycan inhibited FOXP3 expression in both natural Tregs (nTregs) and TGF β -driven adaptive Tregs (aTregs) independent of paracrine Th1, Th2, and Th17 cytokines [104]. While TLR2-induced inhibition of FOXP3 was independent of STAT1, STAT3, STAT4, and STAT6, it was dependent on Myd88 and IRF. Binding of induced IRF1 to IRF1 response elements (IRF-E) in the FOXP3 promoter and intronic enhancers negatively regulated FOXP3 expression to suppress Treg function. Furthermore by inducing divergent chromatin changes at the FOXP3 locus, inflammatory IL-6 and TLR2 signals regulated Treg suppressor function and reduced graft survival in an islet transplantation model. Schroppel's group demonstrated that deficiency of TLR4 in islet graft recipients prolonged graft survival. Low dose rapamycin-treatment of TLR4(-/-) recipients induced permanent and prolonged engraftment of 45% of the islet graft that was dependent on the presence of CD4+CD25+FOXP3+ Tregs [164]. Naïve CD4+CD25⁻ T cells cultured with the TLR4 ligand lipopolysaccharide showed enhanced IL-4, IL-6, IL-17, and IFN- γ secretion and inhibited TGF β -induced FOXP3+Treg generation. These results indicated that inhibition of recipient TLR4 activation at the time of transplantation decreased proinflammatory signals and allowed for Treg generation. Approaches such as generating TLR agonists/antagonists, creating monoclonal antibody to TLRs, blocking key molecules in the signaling pathways and downmodulating TLR signaling may be of immense benefit in the treatment of T1D and islet transplantation.

Other Tolerance Inducing Treatments. Interestingly, treatment of islet graft recipients with bilirubin prolonged islet allograft survival via a Treg-dependent manner in which CD4+CD25+ Treg cells were necessary for tolerance induction and graft acceptance. Bilirubin treatment also promoted *de novo* generation of Tregs possibly accounting for the observed protective effects [165]. A combination treatment of Hmox1 induction, carbon monoxide, and bilirubin administration led to long-term survival and tolerance toward islet allografts by promoting FOXP3+ Tregs and inducing and maintaining tolerance in the recipient [166]. VAG539 is a water soluble derivative of VAF347, a low-molecular-weight compound that activates

the aryl hydrocarbon receptor (AhR). Oral administration of VAG539 promotes long-term graft acceptance and active tolerance in Balb/c mice that receive MHC-mismatched pancreatic islet allograft, resulting in increased frequency of splenic CD4+CD25+FOXP3+ T cells *in vivo* and improved CD4+CD25+FOXP3+ T-cell survival *in vitro* [167]. Interestingly, transfer of CD11c+ DCs but not of CD4+ T or CD19+ B cells, from VAG539-treated long-term tolerant hosts into mice that recently underwent transplantation resulted in donor (C57Bl/6)-specific graft acceptance and in a significantly higher frequency of splenic CD4+CD25+FOXP3+ Tregs. Furthermore, the transfer of these CD4+CD25+ Tregs into recently transplanted mice promoted islet graft acceptance. Also, cell therapy with *in vitro* VAF347-treated bone-marrow-derived mature DCs prevented islet graft rejection, and reduced OVA-specific T-cell responses in OVA-immunized mice. Taken together these data indicate that activation of AhR induces islet allograft-specific tolerance through direct as well as DC-mediated effects on Treg survival and function. Transient depletion of dividing T cells by administration of ganciclovir for 14 days, induced at the time of allogeneic islets transplantation into diabetic transgenic mice that express a thymidine kinase (TK) conditional suicide gene in T cells also resulted in allograft tolerance in 63% of treated mice accompanied by a 2- to 3-fold persistent increase in the proportion of CD4+CD25+FOXP3+ Treg within 3 weeks only in allograft-bearing mice. Additionally, lymphocytes from tolerant mice could transfer tolerance to naïve allografted recipients [168]. Similar results were obtained after cytostatic hydroxyurea treatment in normal mice suggesting that the transient depletion of dividing T cells represented a novel means of immunointervention based on disturbance of T-cell homeostasis and subsequent increase in Treg proportion.

In Vivo and In Vitro Expansion of Tregs. Nagahama et al. established a protocol for *in vivo* and *in vitro* alloantigen-specific expansion of naturally arising CD4+CD25+ regulatory T-cells (Treg) to establish antigen-specific dominant tolerance to allogeneic transplants [169]. They showed that *in vivo* exposure of CD4+CD25+ T cells from normal naïve mice to alloantigen in a T-cell-deficient environment elicited the spontaneous expansion of alloantigen-specific CD4+CD25+ nTregs capable of suppressing allograft rejection mediated by subsequently transferred naïve T cells, leading to long-term graft tolerance. Similarly they demonstrated that antigen-specific expansion of nTregs can be achieved *in vitro* by stimulating CD4+CD25+ T cells from normal animals with alloantigen in the presence of high doses of IL-2. The expanded Tregs were even capable of suppressing secondary mixed leukocyte reaction *in vitro* and, following adoptive transfer, were able to establish antigen-specific long-term graft tolerance. Francis et al. have recently demonstrated that graft-protective Treg arise *in vivo* both from naturally occurring FOXP3+CD4+ Tregs and from non-regulatory FOXP3⁻CD4+ cells [170]. Interestingly, the induction of tolerance also inhibited CD4+ effector cell priming with T cells from tolerant mice demonstrating

impaired effector function *in vitro*. Thus, by converting potential effector cells into graft-protective Tregs and by expanding alloreactive naturally occurring Tregs, adaptive tolerance was induced. Adoptive cell therapy using patient-specific CD4+CD25+ Tregs as individualised medicine to promote clinical transplantation tolerance is very promising [171]. If this principle is to be applied to clinical tolerance induction, strategies targeting potential effector cells will have to be investigated for successful generation of alloreactive Tregs that may be critical for long-term allograft survival without chronic immunosuppression.

The Role of Tolerance-Inducing Dendritic Cell Therapies in Treg and TH17 Cells Interconversion. Activated CD4+ T cells develop into Th1, Th2, or Th17 subsets based on the cytokines they produce and distinct effector functions. Th17 cells produce IL-17A, IL-17F, IL-10, IL-22, and IL-21 and play a role in host defense against infections, and in inducing tissue inflammation in autoimmune disease [172]. The broad distribution profile of IL-17 and IL-22 receptors guarantees induction of a massive tissue reaction. The involvement of differentiation factors (TGF- β plus IL-6 or IL-21), the growth and stabilization factor (IL-23), and the transcription factors (STAT3, ROR γ , and ROR α) in the development and stabilization of Th17 cells has recently been identified [173]. Th17-derived IL-21 plays an important role in the amplification of Th17 cells [8]. Based on the evidence of immunosuppressive TGF- β participation in the differentiation of Th17 cells, the Th17 lineage appears to have a close relationship with CD4+CD25+FOXP3+ Tregs. Th17 cells were shown to be involved in islet transplant rejection, and blockade of IL-23R was shown to positively correlate with reduction in IL-17 expression in a dose-dependent manner [174]. While the combination of anti-CD154 mAb and IL-23R antibody was shown to prevent the acute rejection to some extent, no significant difference was observed when compared with the anti-CD154mAb alone. RelB(lo) DCs generated in the presence of an NF- κ B inhibitor induce Tregs and suppress inflammation. A very important recent study showed that while tolerizing RelB(lo) DCs were able to significantly inhibit diabetes progression when administered to 4-week-old NOD mice, IL-1 β produced in response to islet autoantigen presentation reduced the immunosuppressive capacity of Treg cells and promoted their conversion to Th17 cells [175]. RelB(lo) DCs exacerbated the IL-1-dependent decline in Treg function and promoted Th17 conversion. This study highlights the importance to entertain caution while using tolerizing DC therapies that regulate islet autoantigen priming and prevent diabetes and that progression past the IL-1 β /IL-17 checkpoint signals the need for adapting other tolerizing strategies.

Current Combinatorial Therapeutic Strategies in Clinical Islet Transplantation. Currently, islet transplantation followed by ATG/alemtuzumab (Campath-1H, monoclonal). Anti-CD52 Ab/hOKT3 γ /anti-CD25 (daclizumab) induction therapy along with a sirolimus-based, prednisone-

free maintenance regimen in combination with MMF and low Tacrolimus as well as drugs that demonstrate powerful immunosuppressive/anti-inflammatory potency in the absence of nephrotoxicity and diabetogenicity are under investigation [153, 176–179]. Other drugs currently in Phases II or III of development include Otelixizumab (anti-CD3), Teplizumab (anti-CD3), Rituximab (anti-CD20), Abatacept (CTLA4Ig), DiapPep 277 (heat shock protein), and GAD and Oral Insulin amongst others. GLP-1R agonists like exendin-4 stimulate β -cell proliferation and neogenesis and inhibit β -cell apoptosis while DPPIV inhibitors increase cell insulin content and are therefore of immense benefit in the above mentioned combination therapies for preserving and expanding β -cell mass following transplantation. The activity of potent proinflammatory TNF α can be inhibited by etanercept, a recombinant TNF α receptor protein. A high success rate of insulin independence was achieved using a protocol in which Etanercept was administered as induction therapy following single donor islet transplantation, in combination with Prednisone, Daclizumab, and rabbit ATG [180–182]. Combined treatment with Etanercept and Exenatide in addition to the Edmonton immunosuppressive protocol was shown to reduce the number of islets needed to achieve insulin independence [183] and improve glucose control and graft survival in patients who needed a second transplantation because of progressive graft dysfunction [183, 184]. These combinatorial strategies could also include encapsulation of islets with nanofiber scaffolds or biomatrices or permselective alginate microcapsules synthesized to release immunosuppressive drugs or drugs that stimulate vasculogenesis/angiogenesis to improve transplantation outcomes at extrahepatic sites [185].

2.1.4. Role of Immunomodulatory Stem Cells in Promoting Graft Survival. Several studies indicate that nonimmunogenic multipotent MSCs apart from playing an important role in β -cell replacement therapies owing to their versatile differentiation and *ex vivo* expansion potential, are also cytoprotective immune modulators, exerting therapeutic effects by promoting graft protection, tissue revascularization, and β -cell survival in islet transplantation [186]. Bone-marrow-derived MSCs (BM-MSCs) can enhance repair and regeneration, not only by repopulating damaged tissue, but also by reducing inflammation. Also, they allow transplantation across MHC barriers since they do not possess cell surface human leukocyte antigen (HLA) or MHC class II molecules. Kim et al. evaluated the therapeutic potential of autologous MSCs in preventing graft rejection following allogeneic rat islet transplantation and demonstrated that when combined with cyclosporine A therapy, graft survival attained more than 100 days in 33% of autologous MSCs-plus-CsA-treated recipients [187]. Splenocytes from autologous MSC-plus-CsA-treated rats exhibited a reduced MLR proliferative response to donor stimulators and increased IL-10 release. Interestingly, IL-10 induced by CD11b+ cells and IL-10 activated Tregs played a role in MSC-mediated immune modulation in the rat islet allograft. The authors demonstrated that autologous MSCs-plus-CsA downregulated immune responses and induced

donor-specific T-cell hyporesponsiveness by reducing the production of proinflammatory cytokines and inducing anti-inflammatory cytokine production, especially that of IL-10, during the early post-transplantation period. Tregs contributed at a later phase. Thus, the combined use of autologous MSCs and low-dose CsA exerted a synergistic immunosuppressive effect in an islet allograft model. These results were supported by the demonstration that triple-dose administration of either syngeneic or allogeneic MSCs was able to prevent acute rejection and improve glycemic control in diabetic rats receiving marginal islet mass transplantation via the portal vein [188]. Reduced levels of pro-inflammatory cytokines and glucose as well as low-grade rejections were observed up to 15 days after transplantation indicating the ability of MSCs to prolong graft function by preventing acute rejection. Also the efficacy of MSCs was independent of the administration route and comparable to that of immunosuppressive therapy indicating that MSCs may play an important role in preventing acute rejection and improving graft function in portal vein pancreatic islet transplantation. It is also noteworthy to mention that xenotransplantation of three-dimensional spheroid bodies (SBs) formed under special induction conditions from endometrial mesenchymal stem-like cells (SB-EMSCs) into immunocompromised mice with STZ-induced diabetes restored blood insulin levels to control values and greatly prolonged the survival of graft cells. These results suggest that EMSCs not only played a novel role in the differentiation of pancreatic progenitors, but could also functionally enhance insulin production to restore the regulation of blood glucose levels. It will be interesting to dissect the role of these cells in immunomodulation and vasculogenesis in an *in vivo* transplantation model [189].

The pancreatic endocrine potential of hESC-derived CD34+ cells has recently been demonstrated by Goodrich et al. who transplanted sheep with these cells by *in utero* intraperitoneal injections prior to development of the immune system in the fetus so that tolerance toward foreign antigens was acquired during gestation and persisted in the adult [190]. In animals transplanted with differentiated cell populations and followed up to 55 months after transplantation, they detected human DNA and insulin messenger RNA in sheep pancreases as well as human C-peptide in serum. As few as 23,500 cells were able to achieve long-term sustainable β -cell-like activity. These results along with the absence of teratomas, combined with the hematopoietic potential of these cells suggest that not only do hESC-derived CD34+ cells have potential for long-term *in vivo* endocrine cellular activity but could also be used for the induction of immunological tolerance and bone marrow chimerism prior to cellular therapy for diabetes. A phase II islet transplantation clinical trial at the University of Miami is currently ongoing using monoclonal antibody Campath-1H for induction of immunosuppression combined with the simultaneous infusion of islets with donor CD34+ enriched bone marrow cells (ClinicalTrials.gov identifier: NCT00315614) to reverse hyperglycemia, induce a state of donor-specific tolerance and eliminate the need for continuous immunosuppressive therapy. A study to investigate the immunomodulatory role of mobilized

autologous hematopoietic stem cells (HSCs) via antagonism of the CXCR4-CXCL12 axis in promoting islet engraftment following allotransplantation demonstrated mobilization of HSCs and prolongation of islet graft survival that was further enhanced by the addition of rapamycin to anti-CXCR4 therapy, inducing a robust and transferable host hyporesponsiveness. Mobilized HSCs expressed high levels of the negative costimulatory molecule programmed death ligand 1 (PDL1) and suppressed the *in vitro* alloimmune response. Thus, targeting the CXCR4-CXCL12 axis mobilized autologous HSCs and promoted long-term survival of islet allografts via a PD-L1-mediated mechanism. Halting the CXCR4 antagonist-mediated HSC release by administration of an ACK2 (anti-CD117) mAb restored allograft rejection [191].

The innate anti-inflammatory and immunosuppressive potential of human amniotic epithelial cells (AECs) to create localised immuneprivilege in an *in vitro* islet cell culture system as an alternative to immunosuppressive drug therapy was investigated. Islets transduced with bioengineered cellular constructs composed of human islets and AEC (islet:AEC) demonstrated sustained, physiologically appropriate insulin secretion and reduced mitogen-induced PBL proliferation suggesting that transplanted islets may benefit from the immune-privilege status conferred on them as a consequence of their close proximity to human AEC and that this approach may reduce the need for chronic systemic immunosuppression [192].

2.2. Enhancing Graft Survival by Promoting Vasculogenesis and Reducing Hypoxia. In experimental islet transplantations, both blood perfusion as well as the tissue oxygen tension of the grafted islets are chronically decreased in the transplanted islets, indicating that reestablishment of an appropriate microvascular supply is an essential prerequisite for successful islet engraftment. The islet grafts depend upon endothelial cells and microvessels in the implantation organ for derivation of a new vascular system [193, 194]. Improved islet graft survival and function have been observed on exposure to growth factors such as basic fibroblast growth factor (bFGF), endothelial cell growth factor α and particularly VEGF, which has been known to contribute significantly to the vascularization of transplanted islets [195]. In humans, the VEGF family of homodimeric glycoproteins consists of VEGF-A, -B, -C, -D, and placental growth factor [194]. Interestingly, although pancreatic islets continuously express VEGF-A [196], upon subjection to hypoxia following transplantation devascularized grafted islets significantly increase their expression of VEGF, initiating revascularization and maintaining the vascular permeability [196–199]. VEGF-A stimulates EC permeability and chemotaxis through cognate VEGF receptors and is a prerequisite for islet endothelial fenestration [194–200]. Korsgren and Magnusson's group showed that immobilizing heparin on the islet surface was useful in achieving complete coverage of islets with VEGF-A as a means of attracting ECs to induce angiogenesis and revascularization, ultimately improving islet revascularization and engraftment

in pancreatic islet transplantation [35, 44]. A very recent study indicates that concomitant transplantation of isolated islets with ECs can prolong islet graft survival in diabetic rats [201]. Cartilage oligomeric matrix protein-angiopoietin-1 (COMP-Ang1) is a specific growth factor that induces vascularization via the Tie2 or Tie1 receptor. Using an *in vitro* angiogenesis assay based on a three-dimensional collagen-based culture system, Park et al. recently demonstrated that the transduction of COMP-Ang1 into islets significantly increased angiogenesis [202]. COMP-Ang1 transduced islets also attenuated hyperglycemia in syngeneic STZ-induced diabetic C57BL/6 mice and enhanced glucose tolerance. In another study, following subcutaneous transplantation of BALB/c islets in VEGF and hepatocyte-growth-factor (HGF) supplemented matrigel basement membrane matrix into diabetic *scid* mice [203], histopathologic analysis of the functioning grafts harvested at 15 days revealed significantly increased blood vessel formation and increased number of islets. Enhanced intercellular adhesion molecule (ICAM) and vascular cell adhesion molecule (VCAM) within the islets was also observed suggesting stable blood vessel formation. Transcription factors focal adhesion kinase phosphorylation and extracellular signal-regulated kinase1/2 phosphorylation were also increased (8-fold and 4.6-fold, respectively). These results suggest synergistic enhancement of angiogenesis by VEGF and HGF following islet transplantation resulted in stable engraftment. In order to drive delivery of growth factors such as VEGF and FGF into the dense islet interior, Chow et al. developed heparin-binding peptide amphiphile (HBPA)/heparin nanofiber gels that can activate heparin-binding, angiogenic growth factors [204]. Infiltration of bioactive nanofibers in the interior of islets acted as an artificial extracellular matrix (ECM) improving cell viability and function and enhancing their vascularization in the presence of growth factors such as FGF2 and VEGF. The intraislet nanofibers helped retain FGF2 within the islet for 48 h and increased cell viability significantly for at least 7 days in culture. Furthermore, enhanced insulin secretion was observed with the nanofibers for 3 days in culture. Delivery of FGF2 and VEGF in conjunction with the HBPA/heparin nanofibers also induced a significant amount of islet EC sprouting from the islets into a peptide amphiphile 3D matrix. This approach may have a significant impact on islet transplantation. Mahato coexpressed human VEGF (hVEGF) and human IL-1 receptor antagonist (hIL-1Ra) as well as human HGF (hHGF) and hIL-1Ra in human islets using Adv-hVEGF-hIL-1Ra Adv-hHGF-hIL-1Ra constructs to study their effect on β -cell proliferation and revascularization of islets [205]. A dose- and time-dependent expression of hVEGF and hIL-1Ra or hHGF and hIL-1Ra by islets was observed that led to a decrease in caspase-3 activity and apoptosis induced by a cocktail of TNF- α , IL-1 β , and IFN- γ . Also, transduction of islets with these bipartite Adv vectors prior to transplantation in diabetic NOD*scid* mice reduced blood glucose levels and increased serum insulin and c-peptide levels. Immunohistochemical staining of the graft revealed positivity for human insulin, hVEGF or hHGF, and von Willebrand factor. Transduction with Adv-caspase-3-shRNA also prevented

islets from cytokine-induced apoptosis and improved islet transplantation [205].

The therapeutic potential of hyperbaric oxygen therapy (HBO) in reducing hypoxia, enhancing vessel maturation, and improving engraftment of intraportal islet transplants by promoting angiogenesis in the critical period following transplantation has also been demonstrated [206]. In this study, hyperbaric oxygenation combined with implantation of a foam dressing, vacuum-assisted wound closure (foam+VAC) to create a prevascularized site was used to achieve better results in microencapsulated xenogeneic cell transplantation [206, 207]. Interestingly, following intraportal islet transplantation of porcine islets to diabetic NMRI nu/nu mice, the combination of cytokines with hypoxia resulted in a strong induction of cell death that could be blocked dose-dependently by a selective IKK- β inhibitor that caused systemic NF- κ B inhibition, significantly prolonging islet graft survival [208]. Under hypoxia, NF- κ B activity impaired expression of antiapoptotic genes BCL-xL, c-FLIP and survivin. NF- κ B thus appeared to have an antiapoptotic role under normoxia, while low oxygen conditions decreased its activity and transformed it to a proapoptotic transcription factor in pancreatic islets suggesting that NF- κ B inhibition represented a potential strategy to improve islet transplantation efficiency. Significant islet loss related to reduced survival of large islets compromised by hypoxia has been observed under standard culture conditions. In order to improve the islet graft quality prior to transplantation, rat islets have been cultured for 48 h in a liquid-liquid interface culture system (LICS) using perfluorodecalin, a method of culture that avoids exposure of islets to relative hypoxia [209]. Results indicated that this protocol optimized culture conditions, which preserved both islet viability and significantly increased their ability to engraft successfully after intraportal transplantation and could be used for islet transportation. Evaluating neovascularization and correlating angiogenesis with metabolic and functional islet graft condition in diabetic mice is a crucial aspect in assessing graft survival. To this end, very recent studies demonstrated the successful use of dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) after intravenous injection of gadolinium [210, 211]. MRI has also been used to effectively image isolated mouse islets labeled with novel MRI contrast agent, chitosan-coated superparamagnetic iron oxide (CSPIO) nanoparticles as long as 18 weeks after transplantation as well as islets labeled with Feridex-polyethyleneimine complex [212, 213]. A recent study using a dual-purpose therapy/imaging probe consisting of therapeutic (siRNA targeting apoptosis-related gene human caspase-3) and imaging (magnetic iron oxide nanoparticles, MN) moieties showed that treatment with the probe resulted in significantly better survival of transplanted islets that could be monitored by *in vivo* magnetic resonance imaging (MRI) [214].

2.2.1. Stem Cells in Promoting Angiogenesis/Vasculogenesis. Bone-Marrow-derived stem cells (BMSCs) have been shown to promote islet graft function and survival by initiating

angiogenesis [215, 216]. A recent study involving cotransplantation of bone marrow cells with islets was associated with enhanced islet graft vascularization and function. A significant increase in new peri-islet vessels [216] as well as staining for VEGF was observed. The presence of pancreatic duodenal homeobox-1 (Pdx-1) was detected in BMSCs with an increase in staining over time. Protein array measurements conducted in human islets cocultured with whole human BM for approx. 7 months indicated upregulated levels of angiogenesis factors VEGF-a, PDGF, KGF, TIMP-1, and angiogenin as well as lower protein levels of angiopoietin-2 [215]. Depletion of VEGF-a, eKGF, and PDGF significantly reduced islet vascularization. BM-induced vascularization showed significant EC distribution and islet vascularization was linked to islet growth. Furthermore a 28.66-fold increase in insulin and 24.4-fold glucagon gene expression was also observed. These data indicate that BMSCs induced endocrine cell regeneration via regulation of angiogenesis factors. Transplantation of genetically marked whole bone marrow from Tie2-Cre/ZEG mice into lethally irradiated wild-type mice evoked pronounced proliferation of recipient ECs while significantly increasing β -cell mass and reducing the hyperglycemia of mice subjected to β -cell damage by STZ [217]. A study indicated that bone marrow cells produced nerve growth factor (NGF) and promoted angiogenesis around transplanted islets [218]. Biochemical and histological analyses following syngeneic cotransplantation of islets and bone marrow in STZ-induced diabetic mice indicated significantly low blood glucose levels high serum insulin levels, and increased serum NGF levels. A significant increase in the number of vessels within the graft area at day 14 after transplant was observed along with improvement in graft function [218]. Thus, an important adjuvant role of transplanted BMSCs in both angiogenesis and β -cell regeneration has been elucidated. Cotransplantation of pancreatic islets and adipose-tissue-derived stem cells (ADSCs) too was shown to significantly prolong graft survival and insulin function of islet grafts in diabetic mice. ADSCs have angiogenic potential and anti-inflammatory properties and cotransplantation studies indicated significant revascularization (larger number of von Willebrand factor-positive cells) and marked inhibition of inflammatory cell infiltration, including CD4+ and CD8+ T cells and macrophages, in islets-ADSCs grafts [219]. Creation of a rich subcutaneous vascular network with implanted adipose-tissue-derived stromal cells and adipose tissue enhanced subcutaneous grafting of islets in diabetic mice [220]. Cografting of neural crest stem cells with pancreatic islets in alloxan-induced diabetic mice too improved insulin release and enhanced β -cell proliferation, resulting in increased β -cell mass [221]. Multipotent human MSCs possess powerful *ex vivo* expansion and EC differentiation potential, placing them at the forefront in the field of vasculature-directed cell-based therapy and transplantation. A recent study revealed that islets co-cultured with MSCs demonstrated lower ADP/ATP ratios, higher GSIS indexes and viability. Furthermore, co-cultured islets revealed higher levels of antiapoptotic signal molecules (XIAP, Bcl-xL, Bcl-2, and heat shock protein-32), increased VEGF receptor

2 and Tie-2 mRNA expression and elevated levels of phosphorylated Tie-2 and focal adhesion kinase protein [222]. Islets cultured in MSC-conditioned medium (MSC-CM) for 48 hr significantly lowered blood glucose levels and demonstrated enhanced blood vessel formation upon transplantation into STZ induced diabetic mice. Significant levels of IL-6, IL-8, VEGF-A, HGF, and TGF- β were detected in MSC-CM suggesting that the trophic factors secreted by human MSCs enhanced islet survival and function after transplantation. Similar improvement of islet graft morphology and function attributable in part to the promotion of graft revascularization was observed when Lewis rat islets cocultured with syngeneic MSCs were infused into the liver of STZ-diabetic syngeneic recipients or when islets were cotransplanted under the renal capsule of NOD scid mice with syngeneic MSCs expanded in culture [223]. Other studies have demonstrated increased mean capillary density upon co-transplantation of MSCs with pancreatic islets along with improved islet graft function indicating the beneficial effect of MSC-mediated graft vascularization [224]. In mice, islets co-transplanted with MSCs maintained a morphology closely resembling that of islets in the endogenous pancreas, both in terms of size, and of endocrine and EC distribution. Superior vascular engraftment as shown by increased EC numbers within the endocrine tissue as well as improved graft function demonstrated by normoglycemia achieved in 92% of mice indicated that MSCs profoundly influenced the remodeling process, by improving islet revascularization and maintaining islet organisation [224, 225]. In order to study the effect of MSCs on islet survival and insulin secretion under hypoxia/reoxygenation- (H/R-) induced injury conditions that are associated with islet graft dysfunction, purified rat islets cultured with or without MSCs, were exposed to hypoxia (O₂ \leq 1%) for 8 h followed by reoxygenation for 24 and 48 h, respectively [226]. MSCs maintained a higher level of stimulation index (SI) of GSIS in islets *in vitro*, protected islets from H/R-induced injury by decreasing the apoptotic cell ratio and increasing HIF-1 α , HO-1, and COX-2 mRNA expression, and significantly increased insulin expression following islet transplantation. This study indicated that MSCs could promote anti-apoptotic gene expression by enhancing their resistance to H/R-induced apoptosis and dysfunction providing an experimental basis for the therapeutic use of this strategy for enhancing islet function.

3. Insulin-Secreting β -Cell Generation from Stem Cells for Replacement Therapy in T1D.

Apart from the beneficial therapeutic role of stem cells in immunomodulation and promoting vasculogenesis following islet transplantation, they may also play an important role in regeneration of β -cells. The concept of regenerating β -cells from a self renewing, expandable stock of pluripotent ESCs, pancreas-derived multipotent progenitor/stem cells, extrapancreatic adult stem cells (e.g, BMSCs, neural progenitor cells, UCB-SCs, etc.) or iPSCs into large quantities of cells with an insulin-expressing phenotype *in vitro*

offers an attractive alternative source for β -cell replacement therapy [227, 228]. While the immunosuppressive, anti-inflammatory, and angiogenic properties of MSCs are of tremendous advantage, the ability of iPSCs to generate an unlimited supply of clinically compliant, functional autologous β -cells provides a definitive solution to the cited limitations of islet transplantation, namely, shortage of donor pancreases and the harmful side effects of chronic immunosuppressive therapy.

Based on sequential exposure of human ESCs to epigenetic signals that mimic *in vivo* pancreatic development, insulin-producing cells can be generated from ESCs [227, 229–231]. These differentiated cells display architectural similarity to mature primary islets, are capable of synthesizing insulin, glucagon, somatostatin, pancreatic polypeptide and ghrelin [229, 230], reverse hyperglycemia in diabetic mice, prolong graft survival, and respond successfully to glucose challenge in glucose-tolerant tests (GTT) providing definitive evidence of the ability of hESCs to serve as a renewable source of insulin-secreting β -cells for diabetes cell-replacement therapies. The risk of teratoma formation and tumor formation and the difficulty of purifying the differentiated progeny are major drawbacks. Multipotent BM-MSCs are plastic-adherent cells, expressing surface markers such as CD90, CD73, CD105, CD44, and CD29 that can be isolated and expanded with high efficiency in culture and can differentiate into cells of connective tissue lineages, including bone, fat, cartilage, and muscle [232]. The capacity of BM-MSCs to generate insulin-producing cells [232, 233] capable of producing and releasing insulin in a glucose-dependent manner and normalizing hyperglycemia upon transplantation into a diabetic mice [234, 235] while at the same time abrogating immune injury, altering T cell cytokine pattern toward IL-10/IL-13 production and preserving CD4+/CD8+ FOXP3+ Tregs in the periphery [236] make BM-MSCs an invaluable tool in β -cells replacement therapies. *In vivo* differentiation of hUCB cells into β -cells following transplantation into STZ-induced diabetic immunocompromised [237, 238] or NOD mice [239] indicates the potential role of these cells in β -cell replacement therapy. iPSCs may be derived from autologous somatic cells by ectopic expression of the transcription factors *Oct4*, *Sox2*, *c-myc*, and *Klf4* or *oct3/4*, *sox2*, *nanog*, *lin28* [240] and are molecularly and functionally highly similar to ESCs [241], offering an important alternative to replenish β -cell supply [242] while simultaneously obviating immune concerns such as rejection and chronic immunosuppression. Most human iPSC lines can be induced into Pdx1-positive progenitor cells and further differentiated into pancreatic lineage cells using a stepwise induction strategy [243, 244]. Other alternative sources of β -cells include intrahepatic biliary epithelial cells and gall bladder epithelium [245], human neural progenitor cells [246], hepatic oval cells [247] placenta-derived multipotent stem cells [248], and adult pancreatic stem/progenitor cells [249, 250]. The common embryonic origin of liver and pancreas, similarities in their glucose-sensing systems, mutually expressed transcription factors, and the high level of developmental plasticity exhibited by adult human liver cells indicate the therapeutic potential

of liver stem cells/hepatocytes as a source of pancreatic progenitor tissue. Several studies have demonstrated reprogramming of hepatocytes into function insulin-producing cells by expression of the Pdx1 or its superactive form Pdx1-VP16 fusion protein either alone or in combination with other pancreatic transcription factors using first generation, nontoxic, transiently expressed adenoviral vectors under conditions of hyperglycemia or hepatic regeneration [251–253]. However, although the potential of stem cells in the future of T1D interventional therapies is immense, the accompanying risk of mutagenesis, teratoma and tumor formation needs to be stringently addressed. For now it appears that the combination of multiple therapeutic avenues is required to achieve the dream of permanently reversing/preventing T1D.

4. Conclusion

Areas of current research include the development of less toxic immunosuppressive regimens, the suppression of inflammatory responses immediately following transplantation, the identification of an optimal anatomical site for islet infusion, and the possibility of encapsulating transplanted islets to protect them from the alloimmune response. The generation of Tregs with defined alloantigen specificity could provide dynamic control of rejection responses and offer a potential route to permanent graft survival without the need for life-long nonspecific immunosuppression. Regeneration of β -cells utilizing every kind cell from the pancreas, stem cells as well as cells from alternate sources, followed by transplantation using immunosuppressive regimens that would ensure maximal graft survival through protection from hypoxic and immune insults is an exciting alternative. Clinical islet transplantation represents a possible definitive intervention for patients with T1D and with significant inroads in the branches of stem cell therapy, immunomodulation and gene therapy, the prospect of translating these beneficial interventions into clinical applications that promote successful long-term functional islet graft survival appears within reach.

References

- [1] H. de Kort, E. J. de Koning, T. J. Rabelink, J. A. Bruijn, and I. M. Bajema, "Islet transplantation in type 1 diabetes," *British Medical Journal*, vol. 342, article d217, 2011.
- [2] D. L. Eizirik, M. L. Colli, and F. Ortis, "The role of inflammation in insulinitis and β -cell loss in type 1 diabetes," *Nature Reviews Endocrinology*, vol. 5, no. 4, pp. 219–226, 2009.
- [3] M. Knip and H. Siljander, "Autoimmune mechanisms in type 1 diabetes," *Autoimmunity Reviews*, vol. 7, no. 7, pp. 550–557, 2008.
- [4] J. W. Yoon and H. S. Jun, "Cellular and molecular pathogenic mechanisms of insulin-dependent diabetes mellitus," *Annals of the New York Academy of Sciences*, vol. 928, pp. 200–211, 2001.
- [5] J. W. Yoon, H. S. Jun, and P. Santamaria, "Cellular and molecular mechanisms for the initiation and progression of β cell destruction resulting from the collaboration between

- macrophages and T cells," *Autoimmunity*, vol. 27, no. 2, pp. 109–122, 1998.
- [6] J. W. Yoon and H. S. Jun, "Cellular and molecular roles of β cell autoantigens, macrophages and T cells in the pathogenesis of autoimmune diabetes," *Archives of Pharmacol Research*, vol. 22, no. 5, pp. 437–447, 1999.
- [7] E. Kawasaki, N. Abiru, and K. Eguchi, "Prevention of type 1 diabetes: from the view point of β cell damage," *Diabetes Research and Clinical Practice*, vol. 66, supplement, pp. S27–S32, 2004.
- [8] E. Bettelli, T. Korn, M. Oukka, and V. K. Kuchroo, "Induction and effector functions of TH17 cells," *Nature*, vol. 453, no. 7198, pp. 1051–1057, 2008.
- [9] R. Jain, D. M. Tartar, R. K. Gregg et al., "Innocuous IFN γ induced by adjuvant-free antigen restores normoglycemia in NOD mice through inhibition of IL-17 production," *Journal of Experimental Medicine*, vol. 205, no. 1, pp. 207–218, 2008.
- [10] Y. Zhao, C. Guo, D. Hwang et al., "Selective destruction of mouse islet beta cells by human T lymphocytes in a newly-established humanized type 1 diabetic model," *Biochemical and Biophysical Research Communications*, vol. 399, no. 4, pp. 629–636, 2010.
- [11] D. M. Maahs, N. A. West, J. M. Lawrence, and E. J. Mayer-Davis, "Epidemiology of type 1 diabetes," *Endocrinology and Metabolism Clinics of North America*, vol. 39, no. 3, pp. 481–497, 2010.
- [12] American Diabetes Association, "Diagnosis and classification of diabetes mellitus," *Diabetes Care*, vol. 34, no. 1, pp. S62–S69, 2011.
- [13] X. Huang, D. J. Moore, R. J. Ketchum et al., "Resolving the conundrum of islet transplantation by linking metabolic dysregulation, inflammation, and immune regulation," *Endocrine Reviews*, vol. 29, no. 5, pp. 603–630, 2008.
- [14] A. M. J. Shapiro, J. R. T. Lakey, E. A. Ryan et al., "Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen," *The New England Journal of Medicine*, vol. 343, no. 4, pp. 230–238, 2000.
- [15] A. M. J. Shapiro, C. Ricordi, B. J. Hering et al., "International trial of the Edmonton protocol for islet transplantation," *The New England Journal of Medicine*, vol. 355, no. 13, pp. 1318–1330, 2006.
- [16] R. Alejandro, F. B. Barton, B. J. Hering, and S. Wease, "2008 update from the collaborative islet transplant registry," *Transplantation*, vol. 86, no. 12, pp. 1783–1788, 2008.
- [17] S. Matsumoto, H. Noguchi, M. Takita et al., "ET-Kyoto ductal injection and density-adjusted purification combined with potent anti-inflammatory strategy facilitated single-donor islet transplantation: case reports," *Transplantation Proceedings*, vol. 42, no. 6, pp. 2159–2161, 2010.
- [18] B. J. Hering, R. Kandaswamy, J. D. Ansite et al., "Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes," *Journal of the American Medical Association*, vol. 293, no. 7, pp. 830–835, 2005.
- [19] S. Matsumoto, T. Okitsu, Y. Iwanaga et al., "Follow-up study of the first successful living donor islet transplantation," *Transplantation*, vol. 82, no. 12, pp. 1629–1633, 2006.
- [20] D. E. R. Sutherland, A. C. Gruessner, A. M. Carlson et al., "Islet autotransplant outcomes after total pancreatectomy: a contrast to islet allograft outcomes," *Transplantation*, vol. 86, no. 12, pp. 1799–1802, 2008.
- [21] P. Cravedi, I. M. van der Meer, S. Cattaneo, P. Ruggerenti, and G. Remuzzi, "Successes and disappointments with clinical islet transplantation," *Advances in Experimental Medicine and Biology*, vol. 654, pp. 749–769, 2010.
- [22] P. Fiorina, C. Gremizzi, P. Maffi et al., "Islet transplantation is associated with an improvement of cardiovascular function in type 1 diabetic kidney transplant patients," *Diabetes Care*, vol. 28, no. 6, pp. 1358–1365, 2005.
- [23] P. Fiorina, F. Folli, P. Maffi et al., "Islet transplantation improves vascular diabetic complications in patients with diabetes who underwent kidney transplantation: a comparison between kidney-pancreas and kidney-alone transplantation," *Transplantation*, vol. 75, no. 8, pp. 1296–1301, 2003.
- [24] P. Fiorina, F. Folli, G. Zerbini et al., "Islet transplantation is associated with improvement of renal function among uremic patients with type I diabetes mellitus and kidney transplants," *Journal of the American Society of Nephrology*, vol. 14, no. 8, pp. 2150–2158, 2003.
- [25] R. M. Meloche, "Transplantation for the treatment of type 1 diabetes," *World Journal of Gastroenterology*, vol. 13, no. 47, pp. 6347–6355, 2007.
- [26] R. G. Bretzel, H. Jahr, M. Eckhard, I. Martin, D. Winter, and M. D. Brendel, "Islet cell transplantation today," *Langenbeck's Archives of Surgery*, vol. 392, no. 3, pp. 239–253, 2007.
- [27] R. M. Langer, "Islet transplantation: lessons learned since the Edmonton breakthrough," *Transplantation Proceedings*, vol. 42, no. 5, pp. 1421–1424, 2010.
- [28] H. Noguchi, "Pancreatic islet transplantation," *World Journal of Gastrointestinal Surgery*, vol. 1, no. 1, pp. 16–20, 2009.
- [29] A. N. Balamurugan, R. Bottino, N. Giannoukakis, and C. Smetanka, "Prospective and challenges of islet transplantation for the therapy of autoimmune diabetes," *Pancreas*, vol. 32, no. 3, pp. 231–243, 2006.
- [30] W. E. Winter and D. Schatz, "Prevention strategies for type 1 diabetes mellitus: current status and future directions," *BioDrugs*, vol. 17, no. 1, pp. 39–64, 2003.
- [31] W. Bennet, C. G. Groth, R. Larsson, B. Nilsson, and O. Korsgren, "Isolated human islets trigger an instant blood mediated inflammatory reaction: implications for intraportal islet transplantation as a treatment for patients with type 1 diabetes," *Uppsala Journal of Medical Sciences*, vol. 105, no. 2, pp. 125–133, 2000.
- [32] M. Goto, J. Tjernberg, D. Dufrane et al., "Dissecting the instant blood-mediated inflammatory reaction in islet xenotransplantation," *Xenotransplantation*, vol. 15, no. 4, pp. 225–234, 2008.
- [33] J. Tjernberg, K. N. Ekdahl, J. D. Lambris, O. Korsgren, and B. Nilsson, "Acute antibody-mediated complement activation mediates lysis of pancreatic islets cells and may cause tissue loss in clinical islet transplantation," *Transplantation*, vol. 85, no. 8, pp. 1193–1199, 2008.
- [34] Y. Banz and R. Rieben, "Role of complement and perspectives for intervention in ischemia-reperfusion damage," *Annals of Medicine*. In press.
- [35] S. Cabric, J. Sanchez, T. Lundgren et al., "Islet surface heparinization prevents the instant blood-mediated inflammatory reaction in islet transplantation," *Diabetes*, vol. 56, no. 8, pp. 2008–2015, 2007.
- [36] F. Bertuzzi, S. Marzorati, P. Maffi et al., "Tissue factor and CCL2/monocyte chemoattractant protein-1 released by human islets affect islet engraftment in type 1 diabetic recipients," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 11, pp. 5724–5728, 2004.
- [37] U. Johansson, A. Olsson, S. Gabrielsson, B. Nilsson, and O. Korsgren, "Inflammatory mediators expressed in human islets of Langerhans: implications for islet transplantation,"

- Biochemical and Biophysical Research Communications*, vol. 308, no. 3, pp. 474–479, 2003.
- [38] L. Piemonti, B. E. Leone, R. Nano et al., “Human pancreatic islets produce and secrete MCP-1/CCL2: relevance in human islet transplantation,” *Diabetes*, vol. 51, no. 1, pp. 55–65, 2002.
- [39] L. Moberg, O. Korsgren, and B. Nilsson, “Neutrophilic granulocytes are the predominant cell type infiltrating pancreatic islets in contact with ABO-compatible blood,” *Clinical and Experimental Immunology*, vol. 142, no. 1, pp. 125–131, 2005.
- [40] A. Mita, C. Ricordi, S. Messinger et al., “Antiproinflammatory effects of iodixanol (OptiPrep)-based density gradient purification on human islet preparations,” *Cell Transplantation*, vol. 19, no. 12, pp. 1537–1546, 2010.
- [41] Y. Zhao, J. Xu, J. Wei, J. Li, J. Cai, and G. Miao, “Preservation of islet survival by upregulating $\alpha 3$ integrin signaling: the importance of 3-dimensional islet culture in basement membrane extract,” *Transplantation Proceedings*, vol. 42, no. 10, pp. 4638–4642, 2010.
- [42] U. Johansson, G. Elgue, B. Nilsson, and O. Korsgren, “Composite islet-endothelial cell grafts: a novel approach to counteract innate immunity in islet transplantation,” *American Journal of Transplantation*, vol. 5, no. 11, pp. 2632–2639, 2005.
- [43] H. I. Kim, J. E. Yu, S. Y. Lee et al., “The effect of composite pig islet-human endothelial cell grafts on the instant blood-mediated inflammatory reaction,” *Cell Transplantation*, vol. 18, no. 1, pp. 31–37, 2009.
- [44] U. Johansson, I. Rasmusson, S. P. Niclou et al., “Formation of composite endothelial cell-mesenchymal stem cell islets: a novel approach to promote islet revascularization,” *Diabetes*, vol. 57, no. 9, pp. 2393–2401, 2008.
- [45] N. M. Luan, Y. Teramura, and H. Iwata, “Immobilization of the soluble domain of human complement receptor 1 on agarose-encapsulated islets for the prevention of complement activation,” *Biomaterials*, vol. 31, no. 34, pp. 8847–8853, 2010.
- [46] N. Takemoto, Y. Teramura, and H. Iwata, “Islet surface modification with urokinase through DNA hybridization,” *Bioconjugate Chemistry*, vol. 22, no. 4, pp. 673–678, 2011.
- [47] Y. Teramura and H. Iwata, “Improvement of graft survival by surface modification with poly(ethylene glycol)-lipid and urokinase in intraportal islet transplantation,” *Transplantation*, vol. 91, no. 3, pp. 271–278, 2011.
- [48] K. Tokodai, M. Goto, A. Inagaki et al., “Attenuation of cross-talk between the complement and coagulation cascades by C5a blockade improves early outcomes after intraportal islet transplantation,” *Transplantation*, vol. 90, no. 12, pp. 1358–1365, 2010.
- [49] L. Moberg, A. Olsson, C. Berne et al., “Nicotinamide inhibits tissue factor expression in isolated human pancreatic islets: implications for clinical islet transplantation,” *Transplantation*, vol. 76, no. 9, pp. 1285–1288, 2003.
- [50] C. L. Stabler, X. L. Sun, W. Cui, J. T. Wilson, C. A. Haller, and E. L. Chaikof, “Surface re-engineering of pancreatic islets with recombinant azido-thrombomodulin,” *Bioconjugate Chemistry*, vol. 18, no. 6, pp. 1713–1715, 2007.
- [51] L. Özmen, K. N. Ekdahl, G. Elgue, R. Larsson, O. Korsgren, and B. Nilsson, “Inhibition of thrombin abrogates the instant blood-mediated inflammatory reaction triggered by isolated human islets: possible application of the thrombin inhibitor Melagatran in clinical islet transplantation,” *Diabetes*, vol. 51, no. 6, pp. 1779–1784, 2002.
- [52] W. Bennet, B. Sundberg, T. Lundgren et al., “Damage to porcine islets of Langerhans after exposure to human blood in vitro, or after intraportal transplantation to cynomolgus monkeys: protective effects of sCR1 and heparin,” *Transplantation*, vol. 69, no. 5, pp. 711–719, 2000.
- [53] N. M. Luan, Y. Teramura, and H. Iwata, “Immobilization of soluble complement receptor 1 on islets,” *Biomaterials*, vol. 32, no. 20, pp. 4539–4545, 2011.
- [54] T. Lundgren, “Soluble complement receptor 1 (TP10) preserves adult porcine islet morphology after intraportal transplantation into cynomolgus monkeys,” *Transplantation Proceedings*, vol. 33, no. 1-2, p. 725, 2001.
- [55] C. Beuneu, O. Vosters, Z. Ling et al., “N-Acetylcysteine derivative inhibits procoagulant activity of human islet cells,” *Diabetologia*, vol. 50, no. 2, pp. 343–347, 2007.
- [56] R. B. Elliott, “Towards xenotransplantation of pig islets in the clinic,” *Current Opinion in Organ Transplantation*, vol. 16, no. 2, pp. 195–200, 2011.
- [57] P. J. Cowan and A. J. F. d’Apice, “The coagulation barrier in xenotransplantation: incompatibilities and strategies to overcome them,” *Current Opinion in Organ Transplantation*, vol. 13, no. 2, pp. 178–183, 2008.
- [58] P. J. Cowan, J. C. Roussel, and A. J. F. d’Apice, “The vascular and coagulation issues in xenotransplantation,” *Current Opinion in Organ Transplantation*, vol. 14, no. 2, pp. 161–167, 2009.
- [59] P. J. Cowan, S. C. Robson, and A. J. D’Apice, “Controlling coagulation dysregulation in xenotransplantation,” *Current Opinion in Organ Transplantation*, vol. 16, no. 2, pp. 214–221, 2011.
- [60] Y. Dai, T. D. Vaught, J. Boone et al., “Targeted disruption of the $\alpha 1,3$ -galactosyltransferase gene in cloned pigs,” *Nature Biotechnology*, vol. 20, no. 3, pp. 251–255, 2002.
- [61] C. Koike, R. Kannagi, Y. Takuma et al., “Introduction of $\alpha(1,2)$ -fucosyltransferase and its effect on α -Gal epitopes in transgenic pig,” *Xenotransplantation*, vol. 3, no. 1, pp. 81–86, 1996.
- [62] S. Miyagawa, H. Murakami, Y. Takahagi et al., “Remodeling of the major pig xenoantigen by N-acetylglucosaminyltransferase III in transgenic pig,” *The Journal of Biological Chemistry*, vol. 276, no. 42, pp. 39310–39319, 2001.
- [63] C. J. Phelps, C. Koike, T. D. Vaught et al., “Production of $\alpha 1,3$ -galactosyltransferase-deficient pigs,” *Science*, vol. 299, no. 5605, pp. 411–414, 2003.
- [64] Y. Miwa, K. Yamamoto, A. Onishi et al., “Potential value of human thrombomodulin and DAF expression for coagulation control in pig-to-human xenotransplantation,” *Xenotransplantation*, vol. 17, no. 1, pp. 26–37, 2010.
- [65] C. C. Lin, M. Ezzelarab, H. Hara et al., “Atorvastatin or transgenic expression of TFPI inhibits coagulation initiated by anti-nonGal IgG binding to porcine aortic endothelial cells,” *Journal of Thrombosis and Haemostasis*, vol. 8, no. 9, pp. 2001–2010, 2010.
- [66] N. Klymiuk, B. Aigner, G. Brem, and E. Wolf, “Genetic modification of pigs as organ donors for xenotransplantation,” *Molecular Reproduction and Development*, vol. 77, no. 3, pp. 209–221, 2010.
- [67] D. J. van der Windt, R. Bottino, A. Casu et al., “Long-term controlled normoglycemia in diabetic non-human primates after transplantation with hCD46 transgenic porcine islets,” *American Journal of Transplantation*, vol. 9, no. 12, pp. 2716–2726, 2009.
- [68] L. E. Diamond, C. M. Quinn, M. J. Martin, J. Lawson, J. L. Platt, and J. S. Logan, “A human CD46 transgenic pig model

- system for the study of discordant xenotransplantation," *Transplantation*, vol. 71, no. 1, pp. 132–142, 2001.
- [69] G. A. Langford, N. Yannoutsos, E. Cozzi et al., "Production of pigs transgenic for human decay accelerating factor," *Transplantation Proceedings*, vol. 26, no. 3, pp. 1400–1401, 1994.
- [70] W. L. Fodor, B. L. Williams, L. A. Matis et al., "Expression of a functional human complement inhibitor in a transgenic pig as a model for the prevention of xenogeneic hyperacute organ rejection," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 91, no. 23, pp. 11153–11157, 1994.
- [71] P. Ramirez, M. J. Montoya, A. Ríos et al., "Prevention of hyperacute rejection in a model of orthotopic liver xenotransplantation from pig to baboon using polytransgenic pig livers (CD55, CD59, and H-transferase)," *Transplantation Proceedings*, vol. 37, no. 9, pp. 4103–4106, 2005.
- [72] C. Y. Zhou, E. McInnes, L. Copeman et al., "Transgenic pigs express human CD59, in combination with human membrane cofactor protein and human decay-accelerating factor," *Xenotransplantation*, vol. 12, no. 2, pp. 142–148, 2005.
- [73] B. Eksler and D. K. Cooper, "Overcoming the barriers to xenotransplantation: prospects for the future," *Expert Review of Clinical Immunology*, vol. 6, no. 2, pp. 219–230, 2010.
- [74] P. Schmidt, M. Goto, B. Le Mauff, I. Anegón, and O. Korsgren, "Adenovirus-mediated expression of human CD55 or CD59 protects adult porcine islets from complement-mediated cell lysis by human serum," *Transplantation*, vol. 75, no. 5, pp. 697–702, 2003.
- [75] H. Johansson, A. Lukinius, L. Moberg et al., "Tissue factor produced by the endocrine cells of the islets of langerhans is associated with a negative outcome of clinical islet transplantation," *Diabetes*, vol. 54, no. 6, pp. 1755–1762, 2005.
- [76] P. J. Cowan, A. Aminian, H. Barlow et al., "Protective effects of recombinant human antithrombin III in pig-to-primate renal xenotransplantation," *American Journal of Transplantation*, vol. 2, no. 6, pp. 520–525, 2002.
- [77] Y. Mullen, M. Maruyama, and C. V. Smith, "Current progress and perspectives in immunoisolated islet transplantation," *Journal of Hepato-Biliary-Pancreatic Surgery*, vol. 7, no. 4, pp. 347–357, 2000.
- [78] T. Maki and A. P. Monaco, "Porcine islet xenotransplantation utilizing a vascularized bioartificial pancreas," *Annals of Transplantation*, vol. 2, no. 3, pp. 69–71, 1997.
- [79] F. Wiegand, K. D. Kroncke, and V. Kolb-Bachofen, "Macrophage-generated nitric oxide as cytotoxic factor in destruction of alginate-encapsulated islets: protection by arginine analogs and/or coencapsulated erythrocytes," *Transplantation*, vol. 56, no. 5, pp. 1206–1212, 1993.
- [80] H. Yang, A. Al-Jazaeri, and J. R. Wright Jr., "The immunoprotective effect of sertoli cells coencapsulated with islet xenografts is not dependent upon Fas ligand expression," *Cell Transplantation*, vol. 11, no. 8, pp. 799–801, 2002.
- [81] S. Sigrist, A. Mechine-Neuville, K. Mandes et al., "Influence of VEGF on the viability of encapsulated pancreatic rat islets after transplantation in diabetic mice," *Cell Transplantation*, vol. 12, no. 6, pp. 627–635, 2003.
- [82] K. Bloch, E. Papismedov, K. Yavriyants, M. Vorobeychik, S. Beer, and P. Vardi, "Immobilized microalgal cells as an oxygen supply system for encapsulated pancreatic islets: a feasibility study," *Artificial Organs*, vol. 30, no. 9, pp. 715–718, 2006.
- [83] G. Jourdan, J. Dusseault, P. Y. Benhamou, L. Rosenberg, and J. P. Hallé, "Co-encapsulation of bioengineered IGF-II-producing cells and pancreatic islets: effect on beta-cell survival," *Gene Therapy*, vol. 18, no. 6, pp. 539–545, 2011.
- [84] X. Chen, L. Zhang, Z. Qi et al., "Novel sulfated glucosaminoglycan-alginate microcapsules in islet transplantation: significantly decreased the secretion of monocyte chemoattractant protein 1 and improved the activity of islet in rats," *Transplantation Proceedings*, vol. 41, no. 10, pp. 4307–4312, 2009.
- [85] E. C. Opara, S. H. Mirmalek-Sani, O. Khanna, M. L. Moya, and E. M. Brey, "Design of a bioartificial pancreas," *Journal of Investigative Medicine*, vol. 58, no. 7, pp. 831–837, 2010.
- [86] B. Ludwig, B. Zimerman, A. Steffen et al., "A novel device for islet transplantation providing immune protection and oxygen supply," *Hormone and Metabolic Research*, vol. 42, no. 13, pp. 918–922, 2010.
- [87] T. Meyer, B. Höcht, and K. Ulrichs, "Xenogeneic islet transplantation of microencapsulated porcine islets for therapy of type I diabetes: long-term normoglycemia in STZ-diabetic rats without immunosuppression," *Pediatric Surgery International*, vol. 24, no. 12, pp. 1375–1378, 2008.
- [88] J. Denner, H. J. Schuurman, and C. Patience, "The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—chapter 5: strategies to prevent transmission of porcine endogenous retroviruses," *Xenotransplantation*, vol. 16, no. 4, pp. 239–248, 2009.
- [89] D. Dufrane, R. M. Goebbels, and P. Gianello, "Alginate macroencapsulation of pig islets allows correction of streptozotocin-induced diabetes in primates up to 6 months without immunosuppression," *Transplantation*, vol. 90, no. 10, pp. 1054–1062, 2010.
- [90] R. Calafiore, G. Basta, G. Luca et al., "Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes," *Diabetes Care*, vol. 29, no. 1, pp. 137–138, 2006.
- [91] P. Chhabra, K. Wang, Q. Zeng et al., "Adenosine A2A agonist administration improves islet transplant outcome: evidence for the role of innate immunity in islet graft rejection," *Cell Transplantation*, vol. 19, no. 5, pp. 597–612, 2010.
- [92] N. R. Barshes, S. Wyllie, and J. A. Goss, "Inflammation-mediated dysfunction and apoptosis in pancreatic islet transplantation: implications for intrahepatic grafts," *Journal of Leukocyte Biology*, vol. 77, no. 5, pp. 587–597, 2005.
- [93] C. Gysemans, H. Callewaert, F. Moore et al., "Interferon regulatory factor-1 is a key transcription factor in murine beta cells under immune attack," *Diabetologia*, vol. 52, no. 11, pp. 2374–2384, 2009.
- [94] T. Nitta, T. Itoh, N. Matsuoka et al., "Prevention of early loss of transplanted islets in the liver of mice by adenosine," *Transplantation*, vol. 88, no. 1, pp. 49–56, 2009.
- [95] E. Y. Cheng, V. K. Sharma, C. Chang et al., "Diannexin decreases inflammatory cell infiltration into the islet graft, reduces β -cell apoptosis, and improves early graft function," *Transplantation*, vol. 90, no. 7, pp. 709–716, 2010.
- [96] S. M. Jin, K. S. Kim, S. Y. Lee et al., "The sequential combination of a JNK inhibitor and simvastatin protects porcine islets from peri-transplant apoptosis and inflammation," *Cell Transplantation*. In press.
- [97] H. Zhu, J. Wang, H. Jiang et al., "Bilirubin protects grafts against nonspecific inflammation-induced injury in syngeneic intraportal islet transplantation," *Experimental and Molecular Medicine*, vol. 42, no. 11, pp. 739–748, 2010.
- [98] N. Giannoukakis, W. A. Rudert, M. Trucco, and P. D. Robbins, "Protection of human islets from the effects

- of interleukin- β by adenoviral gene transfer of an I κ B repressor," *The Journal of Biological Chemistry*, vol. 275, no. 47, pp. 36509–36513, 2000.
- [99] P. O. T. Tran, C. E. Gleason, and R. Paul Robertson, "Inhibition of interleukin- β -induced COX-2 and EP3 gene expression by sodium salicylate enhances pancreatic islet β -cell function," *Diabetes*, vol. 51, no. 6, pp. 1772–1778, 2002.
- [100] J. A. Corbett, G. Kwon, M. H. Marino et al., "Tyrosine kinase inhibitors prevent cytokine-induced expression of iNOS and COX-2 by human islets," *American Journal of Physiology*, vol. 270, no. 6, pp. C1581–C1587, 1996.
- [101] F. L. Xiang, X. Lu, B. Strutt, D. J. Hill, and Q. Feng, "NOX2 deficiency protects against streptozotocin-induced β -cell destruction and development of diabetes in mice," *Diabetes*, vol. 59, no. 10, pp. 2603–2611, 2010.
- [102] J. M. Blander and R. Medzhitov, "Toll-dependent selection of microbial antigens for presentation by dendritic cells," *Nature*, vol. 440, no. 7085, pp. 808–812, 2006.
- [103] M. Tilich and R. R. Arora, "Modulation of toll-like receptors by insulin," *American Journal of Therapeutics*. In press.
- [104] G. Lal, N. Yin, J. Xu et al., "Distinct inflammatory signals have physiologically divergent effects on epigenetic regulation of Foxp3 expression and Treg function," *American Journal of Transplantation*, vol. 11, no. 2, pp. 203–214, 2011.
- [105] W. W. C. van Maren, J. F. M. Jacobs, I. J. M. de Vries, S. Nierkens, and G. J. Adema, "Toll-like receptor signalling on Tregs: to suppress or not to suppress?" *Immunology*, vol. 124, no. 4, pp. 445–452, 2008.
- [106] B. Krüger, N. Yin, N. Zhang et al., "Islet-expressed TLR2 and TLR4 sense injury and mediate early graft failure after transplantation," *European Journal of Immunology*, vol. 40, no. 10, pp. 2914–2924, 2010.
- [107] Q. Gao, L. L. Ma, X. Gao, W. Yan, P. Williams, and D. P. Yin, "TLR4 mediates early graft failure after intraportal islet transplantation," *American Journal of Transplantation*, vol. 10, no. 7, pp. 1588–1596, 2010.
- [108] N. Matsuoka, T. Itoh, H. Watarai et al., "High-mobility group box 1 is involved in the initial events of early loss of transplanted islets in mice," *Journal of Clinical Investigation*, vol. 120, no. 3, pp. 735–743, 2010.
- [109] S. G. Rønn, A. Börjesson, C. Bruun et al., "Suppressor of cytokine signalling-3 expression inhibits cytokine-mediated destruction of primary mouse and rat pancreatic islets and delays allograft rejection," *Diabetologia*, vol. 51, no. 10, pp. 1873–1882, 2008.
- [110] G. J. Suo, J. Qin, C. P. Zhong, and Z. X. Zhao, "Suppressor of cytokine signaling 1 inhibits apoptosis of islet grafts through caspase 3 and apoptosis-inducing factor pathways in rats," *Transplantation Proceedings*, vol. 42, no. 7, pp. 2658–2661, 2010.
- [111] M. Solomon, M. Flodström-Tullberg, and N. Sarvetnick, "Beta-cell specific expression of suppressor of cytokine signaling-1 (SOCS-1) delays islet allograft rejection by down-regulating Interferon Regulatory Factor-1 (IRF-1) signaling," *Transplant Immunology*, vol. 24, no. 3, pp. 181–188, 2011.
- [112] J. Emamaullee, P. Liston, R. G. Korneluk, A. M. J. Shapiro, and J. F. Elliott, "XIAP overexpression in islet β -cells enhances engraftment and minimizes hypoxia-reperfusion injury," *American Journal of Transplantation*, vol. 5, no. 6, pp. 1297–1305, 2005.
- [113] A. Plesner, G. Soukhatcheva, R. G. Korneluk, and C. B. Verchere, "XIAP inhibition of β -cell apoptosis reduces the number of islets required to restore euglycemia in a syngeneic islet transplantation model," *Islets*, vol. 2, no. 1, pp. 18–23, 2010.
- [114] H. Dellè and I. L. Noronha, "Induction of indoleamine 2,3-dioxygenase by gene delivery in allogeneic islets prolongs allograft survival," *American Journal of Transplantation*, vol. 10, no. 8, pp. 1918–1924, 2010.
- [115] J. Ye and S. G. Laychock, "A protective role for heme oxygenase expression in pancreatic islets exposed to interleukin- β ," *Endocrinology*, vol. 139, no. 10, pp. 4155–4163, 1998.
- [116] M. M. Ribeiro, D. Klein, A. Pileggi et al., "Heme oxygenase-1 fused to a TAT peptide transduces and protects pancreatic β -cells," *Biochemical and Biophysical Research Communications*, vol. 305, no. 4, pp. 876–881, 2003.
- [117] A. Pileggi, P. Cattani, T. Berney et al., "HO-1 upregulation protects the pancreatic cell line β TC3 from cytokines and Fas-induced apoptosis," *Transplantation Proceedings*, vol. 33, no. 1-2, pp. 266–267, 2001.
- [118] A. Pileggi, R. D. Molano, T. Berney et al., "Heme Oxygenase-1 Induction in Islet Cells Results in Protection from Apoptosis and Improved in Vivo Function after Transplantation," *Diabetes*, vol. 50, no. 9, pp. 1983–1991, 2001.
- [119] S. H. Fu, B. R. S. Hsu, J. H. Juang, S. T. Chen, T. Y. Yang, and S. Hsu, "Cobalt-protoporphyrin treatment enhances murine islets engraftment," *Transplantation Proceedings*, vol. 36, no. 7, pp. 2205–2206, 2004.
- [120] A. Pileggi, R. D. Molano, T. Berney et al., "Prolonged allogeneic islet graft survival by protoporphyrins," *Cell Transplantation*, vol. 14, no. 2-3, pp. 85–96, 2005.
- [121] S. H. Huang, C. H. Chu, J. C. Yu et al., "Transgenic expression of haem oxygenase-1 in pancreatic beta cells protects non-obese mice used as a model of diabetes from autoimmune destruction and prolongs graft survival following islet transplantation," *Diabetologia*, vol. 53, no. 11, pp. 2389–2400, 2010.
- [122] M. M. Sklavos, S. Bertera, H. M. Tse et al., "Redox modulation protects islets from transplant-related injury," *Diabetes*, vol. 59, no. 7, pp. 1731–1738, 2010.
- [123] S. L. Londrigan, R. M. Sutherland, J. L. Brady et al., "In situ protection against islet allograft rejection by CTLA4Ig transduction," *Transplantation*, vol. 90, no. 9, pp. 951–957, 2010.
- [124] J. Zhang, H. Li, N. Jiang et al., "Effects of gene transfer CTLA4Ig and anti-CD40L monoclonal antibody on islet xenograft rejection in mice," *Transplantation Proceedings*, vol. 42, no. 5, pp. 1835–1837, 2010.
- [125] J. Zhang, H. Li, N. Jiang et al., "Inhibition of rejection in murine islet xenografts by CTLA4Ig and CD40L gene transfer," *Chinese Medical Journal*, vol. 123, no. 21, pp. 3106–3109, 2010.
- [126] J. A. Emamaullee, J. Davis, R. Pawlick et al., "Caspase inhibitor therapy synergizes with costimulation blockade to promote indefinite islet allograft survival," *Diabetes*, vol. 59, no. 6, pp. 1469–1477, 2010.
- [127] T. Berney, A. Pileggi, R. D. Molano et al., "The effect of simultaneous CD154 AND LFA-1 blockade on the survival of allogeneic islet grafts in nonobese diabetic mice," *Transplantation*, vol. 76, no. 12, pp. 1669–1674, 2003.
- [128] I. R. Badell, M. C. Russell, P. W. Thompson et al., "LFA-1 - Specific therapy prolongs allograft survival in rhesus macaques," *Journal of Clinical Investigation*, vol. 120, no. 12, pp. 4520–4531, 2010.
- [129] W. Truong, J. C. Plester, W. W. Hancock et al., "Negative and positive co-signaling with anti-BTLA (PJ196) and CTLA4Ig prolongs islet allograft survival," *Transplantation*, vol. 84, no. 10, pp. 1368–1372, 2007.

- [130] C. P. Larsen, T. C. Pearson, A. B. Adams et al., "Rational development of LEA29Y (belatacept), a high-affinity variant of CTLA4-Ig with potent immunosuppressive properties," *American Journal of Transplantation*, vol. 5, no. 3, pp. 443–453, 2005.
- [131] T. H. Watts, "TNF/TNFR family members in costimulation of T cell responses," *Annual Review of Immunology*, vol. 23, pp. 23–68, 2005.
- [132] M. Honkanen-Scott, J. Johnson, B. Hering, and P. Bansal-Pakala, "Blockade of OX40 signals enhance survival of xenografts in spontaneously diabetic NOD mice," *Transplantation Proceedings*, vol. 40, no. 2, pp. 483–485, 2008.
- [133] F. Wang, J. Xia, J. Chen et al., "Combination of antibodies inhibits accelerated rejection mediated by memory T cells in xenograft-primed mice," *Xenotransplantation*, vol. 17, no. 6, pp. 460–468, 2010.
- [134] W. C. Bevier, A. L. Trujillo, G. B. Primbs, M. K. Bradley, and L. Jovanović, "Oral anti-CD3 monoclonal antibody delays diabetes in non-obese diabetic (NOD) mice: effects on pregnancy and offspring—a preliminary report," *Diabetes/Metabolism Research and Reviews*, vol. 27, no. 5, pp. 480–487, 2011.
- [135] B. Keymeulen, E. Vandemeulebroucke, A. G. Ziegler et al., "Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes," *The New England Journal of Medicine*, vol. 352, no. 25, pp. 2598–2608, 2005.
- [136] K. C. Herold, S. E. Gitelman, U. Masharani et al., "A single course of anti-CD3 monoclonal antibody hOKT3γ1 (ala-ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes," *Diabetes*, vol. 54, no. 6, pp. 1763–1769, 2005.
- [137] C. Kuhn, S. You, F. Valette et al., "Human CD3 transgenic mice: preclinical testing of antibodies promoting immune tolerance," *Science Translational Medicine*, vol. 3, no. 68, article 68ra10, 2011.
- [138] B. J. Hering, R. Kandaswamy, J. V. Harmon et al., "Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody," *American Journal of Transplantation*, vol. 4, no. 3, pp. 390–401, 2004.
- [139] H. Ichii and C. Ricordi, "Current status of islet cell transplantation," *Journal of Hepato-Biliary-Pancreatic Surgery*, vol. 16, no. 2, pp. 101–112, 2009.
- [140] J. Xia, J. Chen, W. Shao et al., "Suppressing memory T cell activation induces islet allograft tolerance in alloantigen-primed mice," *Transplant International*, vol. 23, no. 11, pp. 1154–1163, 2010.
- [141] H. Arefanian, E. B. Tredget, R. V. Rajotte, R. G. Gill, G. S. Korbutt, and G. R. Rayat, "Short-term administrations of a combination of anti-LFA-1 and anti-CD154 monoclonal antibodies induce tolerance to neonatal porcine islet xenografts in mice," *Diabetes*, vol. 59, no. 4, pp. 958–966, 2010.
- [142] J. Xia, J. Chen, W. Shao et al., "Suppressing memory T cell activation induces islet allograft tolerance in alloantigen-primed mice," *Transplant International*, vol. 23, no. 11, pp. 1154–1163, 2010.
- [143] F. Wang, J. Xia, J. Chen et al., "Combination of antibodies inhibits accelerated rejection mediated by memory T cells in xenograft-primed mice," *Xenotransplantation*, vol. 17, no. 6, pp. 460–468, 2010.
- [144] H. M. McGuire, S. Walters, A. Vogelzang et al., "Interleukin-21 is critically required in autoimmune and allogeneic responses to islet tissue in murine models," *Diabetes*, vol. 60, no. 3, pp. 867–875, 2011.
- [145] A. Li, J. Chen, M. Hattori et al., "A therapeutic DNA vaccination strategy for autoimmunity and transplantation," *Vaccine*, vol. 28, no. 8, pp. 1897–1904, 2010.
- [146] E. Pinto, B. Zhang, S. Song, N. Bodor, P. Buchwald, and G. Hochhaus, "Feasibility of localized immunosuppression: 2. PLA microspheres for the sustained local delivery of a soft immunosuppressant," *Pharmazie*, vol. 65, no. 6, pp. 429–435, 2010.
- [147] P. Buchwald, N. Bocca, S. Marzorati et al., "Feasibility of localized immunosuppression: 1. Exploratory studies with glucocorticoids in a biohybrid device designed for cell transplantation," *Pharmazie*, vol. 65, no. 6, pp. 421–428, 2010.
- [148] K. J. Wood, H. Ushigome, M. Karim et al., "Regulatory cells in transplantation," *Novartis Foundation Symposium*, vol. 252, pp. 177–193, 2003.
- [149] J. F. Magliocca and S. J. Knechtle, "The evolving role of alemtuzumab (Campath-1H) for immunosuppressive therapy in organ transplantation," *Transplant International*, vol. 19, no. 9, pp. 705–714, 2006.
- [150] M. Lopez, M. R. Clarkson, M. Albin, M. H. Sayegh, and N. Najafian, "A novel mechanism of action for anti-thymocyte globulin: induction of CD4+CD25+Foxp3+ regulatory T cells," *Journal of the American Society of Nephrology*, vol. 17, no. 10, pp. 2844–2853, 2006.
- [151] A. Vergani, F. D'Addio, M. Jurewicz et al., "A novel clinically relevant strategy to abrogate autoimmunity and regulate alloimmunity in NOD mice," *Diabetes*, vol. 59, no. 9, pp. 2253–2264, 2010.
- [152] C. Liu, H. Noorchashm, J. A. Sutter et al., "B lymphocyte-directed immunotherapy promotes long-term islet allograft survival in nonhuman primates," *Nature Medicine*, vol. 13, no. 11, pp. 1295–1298, 2007.
- [153] A. M. Posselt, G. L. Szot, L. A. Frassetto et al., "Islet transplantation in type 1 diabetic patients using calcineurin inhibitor-free immunosuppressive protocols based on T-cell adhesion or costimulation blockade," *Transplantation*, vol. 90, no. 12, pp. 1595–1601, 2010.
- [154] K. L. Pothoven, T. Kheradmand, Q. Yang et al., "Rapamycin-conditioned donor dendritic cells differentiate CD4+CD25+Foxp3+ T cells in vitro with TGF-β1 for islet transplantation," *American Journal of Transplantation*, vol. 10, no. 8, pp. 1774–1784, 2010.
- [155] D. R. Tonkin and K. Haskins, "Regulatory T cells enter the pancreas during suppression of type 1 diabetes and inhibit effector T cells and macrophages in a TGF-β-dependent manner," *European Journal of Immunology*, vol. 39, no. 5, pp. 1313–1322, 2009.
- [156] R. Melzi, P. Maffi, R. Nano et al., "Rapamycin does not adversely affect intrahepatic islet engraftment in mice and improves early islet engraftment in humans," *Islets*, vol. 1, no. 1, pp. 42–49, 2009.
- [157] Y. D. Muller, G. Mai, P. Morel et al., "Anti-CD154 mAb and rapamycin induce T regulatory cell mediated tolerance in rat-to-mouse islet transplantation," *PLoS ONE*, vol. 5, no. 4, Article ID e10352, 2010.
- [158] P. Monti, M. Scirpoli, P. Maffi et al., "Rapamycin monotherapy in patients with type 1 diabetes modifies CD4+CD25+FOXP3+ Regulatory T-Cells," *Diabetes*, vol. 57, no. 9, pp. 2341–2347, 2008.
- [159] X. X. Zheng, A. Sánchez-Fueyo, M. Sho, C. Domenig, M. H. Sayegh, and T. B. Strom, "Favorably tipping the balance between cytopathic and regulatory T cells to create transplantation tolerance," *Immunity*, vol. 19, no. 4, pp. 503–514, 2003.

- [160] W. Zhang, D. Zhang, M. Shen et al., "Combined administration of a mutant TGF- β 1/Fc and rapamycin promotes induction of regulatory T cells and islet allograft tolerance," *Journal of Immunology*, vol. 185, no. 8, pp. 4750–4759, 2010.
- [161] C. Pasare and R. Medzhitov, "Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells," *Science*, vol. 299, no. 5609, pp. 1033–1036, 2003.
- [162] M. B. Torchinsky, J. Garaude, A. P. Martin, and J. M. Blander, "Innate immune recognition of infected apoptotic cells directs T H17 cell differentiation," *Nature*, vol. 458, no. 7234, pp. 78–82, 2009.
- [163] L. Chen, E. Ahmed, T. Wang et al., "TLR Signals promote IL-6/IL-17-dependent transplant rejection," *Journal of Immunology*, vol. 182, no. 10, pp. 6217–6225, 2009.
- [164] N. Zhang, B. Krüger, G. Lal et al., "Inhibition of TLR4 signaling prolongs Treg-dependent murine islet allograft survival," *Immunology Letters*, vol. 127, no. 2, pp. 119–125, 2010.
- [165] F. Rocuts, X. Zhang, J. Yan et al., "Bilirubin promotes de novo generation of T regulatory cells," *Cell Transplantation*, vol. 19, no. 4, pp. 443–451, 2010.
- [166] S. S. Lee, W. Gao, S. Mazzola et al., "Heme oxygenase-1, carbon monoxide, and bilirubin induce tolerance in recipients toward islet allografts by modulating T regulatory cells," *FASEB Journal*, vol. 21, no. 13, pp. 3450–3457, 2007.
- [167] E. Hauben, S. Gregori, E. Draghici et al., "Activation of the aryl hydrocarbon receptor promotes allograft-specific tolerance through direct and dendritic cell-mediated effects on regulatory T cells," *Blood*, vol. 112, no. 4, pp. 1214–1222, 2008.
- [168] S. Giraud, B. Barrou, S. Sebillaud, P. Debré, D. Klatzmann, and V. Thomas-Vaslin, "Transient depletion of dividing T lymphocytes in mice induces the emergence of regulatory T cells and dominant tolerance to islet allografts," *American Journal of Transplantation*, vol. 8, no. 5, pp. 942–953, 2008.
- [169] K. Nagahama, E. Nishimura, and S. Sakaguchi, "Induction of tolerance by adoptive transfer of treg cells," *Methods in Molecular Biology*, vol. 380, pp. 431–442, 2007.
- [170] R. S. Francis, G. Feng, T. Tha-In, I. S. Lyons, K. J. Wood, and A. Bushell, "Induction of transplantation tolerance converts potential effector T cells into graft-protective regulatory T cells," *European Journal of Immunology*, vol. 41, no. 3, pp. 726–738, 2011.
- [171] S. Jiang, R. I. Lechler, X. S. He, and J. F. Huang, "CD4+CD25+ regulatory T cell therapy for the induction of donor-specific clinical transplantation tolerance," *Expert Opinion on Biological Therapy*, vol. 6, no. 10, pp. 1003–1009, 2006.
- [172] M. B. Torchinsky and J. M. Blander, "T helper 17 cells: discovery, function, and physiological trigger," *Cellular and Molecular Life Sciences*, vol. 67, no. 9, pp. 1407–1421, 2010.
- [173] T. Korn, E. Bettelli, M. Oukka, and V. K. Kuchroo, "IL-17 and Th17 cells," *Annual Review of Immunology*, vol. 27, pp. 485–517, 2009.
- [174] J. J. Xiong, H. M. Lu, X. J. Du, N. W. Ke, and W. M. Hu, "Study on the role of Th17 cells in the islet transplantation," *Sichuan Da Xue Xue Bao Yi Xue Ban*, vol. 41, no. 4, pp. 638–643, 2010.
- [175] S. Bertin-Maghit, D. Pang, B. O'Sullivan et al., "Interleukin-1 β produced in response to islet autoantigen presentation differentiates T-helper 17 cells at the expense of regulatory T-cells: implications for the timing of tolerizing immunotherapy," *Diabetes*, vol. 60, no. 1, pp. 248–257, 2011.
- [176] D. Mineo, J. Sageshima, G. W. Burke, and C. Ricordi, "Minimization and withdrawal of steroids in pancreas and islet transplantation," *Transplant International*, vol. 22, no. 1, pp. 20–37, 2009.
- [177] X. Luo, K. C. Herold, and S. D. Miller, "Immunotherapy of type 1 diabetes: where are we and where should we be going?" *Immunity*, vol. 32, no. 4, pp. 488–499, 2010.
- [178] L. Li, Z. Yi, R. Tisch, and B. Wang, "Immunotherapy of type 1 diabetes," *Archivum Immunologiae et Therapiae Experimentalis*, vol. 56, no. 4, pp. 227–236, 2008.
- [179] C. Y. Du and E. K. Xu, "Protective effect of glucocorticoid-free immunosuppressive regimen in allogenic islet transplantation," *Hepatobiliary and Pancreatic Diseases International*, vol. 5, no. 1, pp. 43–47, 2006.
- [180] M. D. Bellin, R. Kandaswamy, J. Parkey et al., "Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes," *American Journal of Transplantation*, vol. 8, no. 11, pp. 2463–2470, 2008.
- [181] P. Gillard, B. Keymeulen, and C. Mathieu, "Beta-cell transplantation in type 1 diabetic patients: a work in progress to cure," *Verhandelungen—Koninklijke Academie voor Geneeskunde van België*, vol. 72, no. 1-2, pp. 71–98, 2010.
- [182] A. Gangemi, P. Salehi, B. Hatipoglu et al., "Islet transplantation for brittle type 1 diabetes: the UIC protocol," *American Journal of Transplantation*, vol. 8, no. 6, pp. 1250–1261, 2008.
- [183] R. N. Faradji, T. Tharavani, S. Messinger et al., "Long-term insulin independence and improvement in insulin secretion after supplemental islet infusion under exenatide and etanercept," *Transplantation*, vol. 86, no. 12, pp. 1658–1665, 2008.
- [184] K. A. Ghofaili, M. Fung, Z. Ao et al., "Effect of exenatide on β cell function after islet transplantation in type 1 diabetes," *Transplantation*, vol. 83, no. 1, pp. 24–28, 2007.
- [185] T. Froud, R. N. Faradji, A. Pileggi et al., "The use of exenatide in islet transplant recipients with chronic allograft dysfunction: safety, efficacy, and metabolic effects," *Transplantation*, vol. 86, no. 1, pp. 36–45, 2008.
- [186] V. Sordi and L. Piemonti, "Mesenchymal stem cells as feeder cells for pancreatic islet transplants," *Review of Diabetic Studies*, vol. 7, no. 2, pp. 132–143, 2010.
- [187] Y. H. Kim, Y. M. Wee, M. Y. Choi, D. G. Lim, S. C. Kim, and D. J. Han, "IL-10 induced by CD11b+ cells and IL-10 activated regulatory T cells play a role in immune modulation of mesenchymal stem cells in rat islet allograft," *Journal of Molecular Medicine*. In press.
- [188] B. Longoni, E. Szilagy, P. Quaranta et al., "Mesenchymal stem cells prevent acute rejection and prolong graft function in pancreatic islet transplantation," *Diabetes Technology & Therapeutics*, vol. 12, no. 6, pp. 435–446, 2010.
- [189] H. Y. Li, Y. J. Chen, S. J. Chen et al., "Induction of insulin-producing cells derived from endometrial mesenchymal stem-like cells," *Journal of Pharmacology and Experimental Therapeutics*, vol. 335, no. 3, pp. 817–829, 2010.
- [190] A. D. Goodrich, A. Ersek, N. M. Varain et al., "In vivo generation of β -cell-like cells from CD34+ cells differentiated from human embryonic stem cells," *Experimental Hematology*, vol. 38, no. 6, pp. 516–525.e4, 2010.
- [191] P. Fiorina, M. Jurewicz, A. Vergani et al., "Targeting the CXCR4-CXCL12 axis mobilizes autologous hematopoietic stem cells and prolongs islet allograft survival via programmed death ligand 1," *Journal of Immunology*, vol. 186, no. 1, pp. 121–131, 2011.
- [192] K. M. Qureshi, R. J. Oliver, M. B. Paget, H. E. Murray, C. J. Bailey, and R. Downing, "Human amniotic epithelial cells induce localised cell mediated immune-privilege in

- vitro: implications for pancreatic islet transplantation,” *Cell Transplantation*. In press.
- [193] L. Jansson and P. O. Carlsson, “Graft vascular function after transplantation of pancreatic islets,” *Diabetologia*, vol. 45, no. 6, pp. 749–763, 2002.
- [194] N. Ferrara, H. P. Gerber, and J. LeCouter, “The biology of VEGF and its receptors,” *Nature Medicine*, vol. 9, no. 6, pp. 669–676, 2003.
- [195] M. Shimoda, S. Chen, H. Noguchi, S. Matsumoto, and P. A. Grayburn, “In vivo non-viral gene delivery of human vascular endothelial growth factor improves revascularisation and restoration of euglycaemia after human islet transplantation into mouse liver,” *Diabetologia*, vol. 53, no. 8, pp. 1669–1679, 2010.
- [196] G. Christofori, P. Naik, and D. Hanahan, “Vascular endothelial growth factor and its receptors, flt-1 and flk-1, are expressed in normal pancreatic islets and throughout islet cell tumorigenesis,” *Molecular Endocrinology*, vol. 9, no. 12, pp. 1760–1770, 1995.
- [197] B. Vasir, L. P. Aiello, K. H. Yoon, R. R. Quicquel, S. Bonner-Weir, and G. C. Weir, “Hypoxia induces vascular endothelial growth factor gene and protein expression in cultured rat islet cells,” *Diabetes*, vol. 47, no. 12, pp. 1894–1903, 1998.
- [198] D. L. Gorden, S. J. Mandriota, R. Montesano, L. Orci, and M. S. Pepper, “Vascular endothelial growth factor is increased in devascularized rat islets of Langerhans in vitro,” *Transplantation*, vol. 63, no. 3, pp. 436–443, 1997.
- [199] E. Lammert, G. Gu, M. McLaughlin et al., “Role of VEGF-A in vascularization of pancreatic islets,” *Current Biology*, vol. 13, no. 12, pp. 1070–1074, 2003.
- [200] W. G. Roberts and G. E. Palade, “Increased microvascular permeability and endothelial fenestration induced by vascular endothelial growth factor,” *Journal of Cell Science*, vol. 108, no. 6, pp. 2369–2379, 1995.
- [201] H. J. Song, W. J. Xue, Y. Li et al., “Prolongation of islet graft survival using concomitant transplantation of islets and vascular endothelial cells in diabetic rats,” *Transplantation Proceedings*, vol. 42, no. 7, pp. 2662–2665, 2010.
- [202] K. S. Park, E. Y. Shim, B. K. Choi et al., “Cartilage oligomeric matrix protein-angiopoietin-1 enhances angiogenesis of isolated islet and maintains normoglycemia following transplantation,” *Transplantation Proceedings*, vol. 42, no. 7, pp. 2653–2657, 2010.
- [203] A. Golocheikine, V. Tiriveedhi, N. Angaswamy, N. Benschoff, R. Sabarinathan, and T. Mohanakumar, “Cooperative signaling for angiogenesis and neovascularization by VEGF and HGF following islet transplantation,” *Transplantation*, vol. 90, no. 7, pp. 725–731, 2010.
- [204] L. W. Chow, L. J. Wang, D. B. Kaufman, and S. I. Stupp, “Self-assembling nanostructures to deliver angiogenic factors to pancreatic islets,” *Biomaterials*, vol. 31, no. 24, pp. 6154–6161, 2010.
- [205] R. I. Mahato, “Gene expression and silencing for improved islet transplantation,” *Journal of Controlled Release*, vol. 140, no. 3, pp. 262–267, 2009.
- [206] N. Sakata, N. K. Chan, R. P. Ostrowski et al., “Hyperbaric oxygen therapy improves early posttransplant islet function,” *Pediatric Diabetes*, vol. 11, no. 7, pp. 471–478, 2010.
- [207] P. Stiegler, V. Matzi, E. Pierer et al., “Creation of a prevascularized site for cell transplantation in rats,” *Xenotransplantation*, vol. 17, no. 5, pp. 379–390, 2010.
- [208] C. Chen, R. Moreno, B. Samikannu, R. G. Bretzel, M. L. Schmitz, and T. Linn, “Improved intraportal islet transplantation outcome by systemic IKK-beta inhibition: NF- κ B activity in pancreatic islets depends on oxygen availability,” *American Journal of Transplantation*, vol. 11, no. 2, pp. 215–224, 2011.
- [209] M. T. Juszczak, A. Elsadig, A. Kumar et al., “Use of perfluorodecalin for pancreatic islet culture prior to transplantation: a liquid-liquid interface culture system-preliminary report,” *Cell Transplantation*, vol. 20, no. 2, pp. 323–332, 2011.
- [210] N. Sakata, A. Obenaus, N. K. Chan, P. Hayes, J. Chrisler, and E. Hathout, “Correlation between angiogenesis and islet graft function in diabetic mice: magnetic resonance imaging assessment,” *Journal of Hepato-Biliary-Pancreatic Sciences*, vol. 17, no. 5, pp. 692–700, 2010.
- [211] N. Chan, A. Obenaus, A. Tan et al., “Monitoring neovascularization of intraportal islet grafts by dynamic contrast enhanced magnetic resonance imaging,” *Islets*, vol. 1, no. 3, pp. 249–255, 2009.
- [212] J. H. Juang, J. J. Wang, C. R. Shen et al., “Magnetic resonance imaging of transplanted mouse islets labeled with chitosan-coated super para-magnetic iron oxide nanoparticles,” *Transplantation Proceedings*, vol. 42, no. 6, pp. 2104–2148, 2009.
- [213] S. Zhang, H. He, W. Lu, Q. Xu, B. Zhou, and X. Tang, “Tracking intrahepatically transplanted islets labeled with Feridex-polyethyleneimine complex using a clinical 3.0-T magnetic resonance imaging scanner,” *Pancreas*, vol. 38, no. 3, pp. 293–302, 2009.
- [214] P. Wang, M. V. Yigit, Z. Medarova et al., “Combined small interfering RNA therapy and in vivo magnetic resonance imaging in islet transplantation,” *Diabetes*, vol. 60, no. 2, pp. 565–571, 2011.
- [215] J. Z. Q. Luo, F. Xiong, A. S. Al-Homsi, T. Roy, and L. G. Luo, “Human BM stem cells initiate angiogenesis in human islets in vitro,” *Bone Marrow Transplantation*. In press.
- [216] N. Sakata, N. K. Chan, J. Chrisler, A. Obenaus, and E. Hathout, “Bone marrow cell cotransplantation with islets improves their vascularization and function,” *Transplantation*, vol. 89, no. 6, pp. 686–693, 2010.
- [217] A. H. Rosengren, J. Taneera, S. Rymo, and E. Renström, “Bone marrow transplantation stimulates pancreatic β -cell replication after tissue damage,” *Islets*, vol. 1, no. 1, pp. 10–18, 2009.
- [218] N. Sakata, N. K. Chan, J. Chrisler, A. Obenaus, and E. Hathout, “Bone marrow cells produce nerve growth factor and promote angiogenesis around transplanted islets,” *World Journal of Gastroenterology*, vol. 16, no. 10, pp. 1215–1220, 2010.
- [219] Y. Ohmura, M. Tanemura, N. Kawaguchi et al., “Combined transplantation of pancreatic islets and adipose tissue-derived stem cells enhances the survival and insulin function of islet grafts in diabetic mice,” *Transplantation*, vol. 90, no. 12, pp. 1366–1373, 2010.
- [220] Y. Fumimoto, A. Matsuyama, H. Komoda et al., “Creation of a rich subcutaneous vascular network with implanted adipose tissue-derived stromal cells and adipose tissue enhances subcutaneous grafting of islets in diabetic mice,” *Tissue Engineering Part C*, vol. 15, no. 3, pp. 437–444, 2009.
- [221] J. Olerud, N. Kanaykina, S. Vasilovska et al., “Neural crest stem cells increase beta cell proliferation and improve islet function in co-transplanted murine pancreatic islets,” *Diabetologia*, vol. 52, no. 12, pp. 2594–2601, 2009.
- [222] K. S. Park, Y. S. Kim, J. H. Kim et al., “Trophic molecules derived from human mesenchymal stem cells enhance survival, function, and angiogenesis of isolated islets after

- transplantation," *Transplantation*, vol. 89, no. 5, pp. 509–517, 2010.
- [223] T. Ito, S. Itakura, I. Todorov et al., "Mesenchymal stem cell and islet co-transplantation promotes graft revascularization and function," *Transplantation*, vol. 89, no. 12, pp. 1438–1445, 2010.
- [224] M. Figliuzzi, R. Cornolti, N. Perico et al., "Bone marrow-derived mesenchymal stem cells improve islet graft function in diabetic rats," *Transplantation Proceedings*, vol. 41, no. 5, pp. 1797–1800, 2009.
- [225] C. L. Rackham, P. C. Chagastelles, N. B. Nardi, A. C. Hauge-Evans, P. M. Jones, and A. J.F. King, "Co-transplantation of mesenchymal stem cells maintains islet organisation and morphology in mice," *Diabetologia*, vol. 54, no. 5, pp. 1127–1135, 2011.
- [226] Y. Lu, X. Jin, Y. Chen et al., "Mesenchymal stem cells protect islets from hypoxia/reoxygenation-induced injury," *Cell Biochemistry and Function*, vol. 28, no. 8, pp. 637–643, 2010.
- [227] C. Aguayo-Mazzucato and S. Bonner-Weir, "Stem cell therapy for type 1 diabetes mellitus," *Nature Reviews Endocrinology*, vol. 6, no. 3, pp. 139–148, 2010.
- [228] M. E. Furth and A. Atala, "Stem cell sources to treat diabetes," *Journal of Cellular Biochemistry*, vol. 106, no. 4, pp. 507–511, 2009.
- [229] K. A. D'Amour, A. G. Bang, S. Eliazar et al., "Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells," *Nature Biotechnology*, vol. 24, no. 11, pp. 1392–1401, 2006.
- [230] E. Kroon, L. A. Martinson, K. Kadoya et al., "Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo," *Nature Biotechnology*, vol. 26, no. 4, pp. 443–452, 2008.
- [231] T. Guo and M. Hebrok, "Stem cells to pancreatic beta-cells: new sources for diabetes cell therapy," *Endocrine Reviews*, vol. 30, no. 3, pp. 214–227, 2009.
- [232] V. Volarevic, N. Arsenijevic, M. L. Lukic, and M. Stojkovic, "Concise review: mesenchymal stem cell treatment of the complications of diabetes mellitus," *Stem Cells*, vol. 29, no. 1, pp. 5–10, 2011.
- [233] Q. P. Xie, H. Huang, B. Xu et al., "Human bone marrow mesenchymal stem cells differentiate into insulin-producing cells upon microenvironmental manipulation in vitro," *Differentiation*, vol. 77, no. 5, pp. 483–491, 2009.
- [234] L. B. Chen, X. B. Jiang, and L. Yang, "Differentiation of rat marrow mesenchymal stem cells into pancreatic islet beta-cells," *World Journal of Gastroenterology*, vol. 10, no. 20, pp. 3016–3020, 2004.
- [235] O. Karnieli, Y. Izhar-Prato, S. Bulvik, and S. Efrat, "Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation," *Stem Cells*, vol. 25, no. 11, pp. 2837–2844, 2007.
- [236] I. Boumaza, S. Srinivasan, W. T. Witt et al., "Autologous bone marrow-derived rat mesenchymal stem cells promote PDX-1 and insulin expression in the islets, alter T cell cytokine pattern and preserve regulatory T cells in the periphery and induce sustained normoglycemia," *Journal of Autoimmunity*, vol. 32, no. 1, pp. 33–42, 2009.
- [237] V. S. Parekh, M. V. Joglekar, and A. A. Hardikar, "Differentiation of human umbilical cord blood-derived mononuclear cells to endocrine pancreatic lineage," *Differentiation*, vol. 78, no. 4, pp. 232–240, 2009.
- [238] Y. Zhao, B. Lin, M. Dingeldein, C. Guo, D. Hwang, and M. J. Holterman, "New type of human blood stem cell: a double-edged sword for the treatment of type 1 diabetes," *Translational Research*, vol. 155, no. 5, pp. 211–216, 2010.
- [239] T. Koblas, K. Zacharovová, Z. Berková et al., "In vivo differentiation of human umbilical cord blood-derived cells into insulin-producing β cells," *Folia Biologica*, vol. 55, no. 6, pp. 224–232, 2009.
- [240] K. Hochedlinger and K. Plath, "Epigenetic reprogramming and induced pluripotency," *Development*, vol. 136, no. 4, pp. 509–523, 2009.
- [241] M. Baker, "Stem cells: fast and furious," *Nature*, vol. 458, no. 7241, pp. 962–965, 2009.
- [242] K. Tateishi, J. He, O. Taranova, G. Liang, A. C. D'Alessio, and Y. Zhang, "Generation of insulin-secreting islet-like clusters from human skin fibroblasts," *The Journal of Biological Chemistry*, vol. 283, no. 46, pp. 31601–31607, 2008.
- [243] T. Thatava, T. J. Nelson, R. Edukulla et al., "Indolactam V/GLP-1-mediated differentiation of human iPS cells into glucose-responsive insulin-secreting progeny," *Gene Therapy*, vol. 18, no. 3, pp. 283–293, 2011.
- [244] D. Zhang, W. Jiang, M. Liu et al., "Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells," *Cell Research*, vol. 19, no. 4, pp. 429–438, 2009.
- [245] S. Sahu, D. Tosh, and A. A. Hardikar, "New sources of β -cells for treating diabetes," *Journal of Endocrinology*, vol. 202, no. 1, pp. 13–16, 2009.
- [246] Y. Hori, X. Gu, X. Xie, and S. K. Kim, "Differentiation of insulin-producing cells from human neural progenitor cells," *PLoS Medicine*, vol. 2, no. 4, article e103, 2005.
- [247] S. Kim, J. S. Shin, H. J. Kim, R. C. Fisher, M. J. Lee, and C. W. Kim, "Streptozotocin-induced diabetes can be reversed by hepatic oval cell activation through hepatic transdifferentiation and pancreatic islet regeneration," *Laboratory Investigation*, vol. 87, no. 7, pp. 702–712, 2007.
- [248] C. M. Chang, C. L. Kao, Y. L. Chang et al., "Placenta-derived multipotent stem cells induced to differentiate into insulin-positive cells," *Biochemical and Biophysical Research Communications*, vol. 357, no. 2, pp. 414–420, 2007.
- [249] F. Carlotti, A. Zaldumbide, C. J. Loomans et al., "Isolated human islets contain a distinct population of mesenchymal stem cells," *Islets*, vol. 2, no. 3, pp. 164–173, 2010.
- [250] A. Suzuki, H. Nakauchi, and H. Taniguchi, "Prospective isolation of multipotent pancreatic progenitors using flow-cytometric cell sorting," *Diabetes*, vol. 53, no. 8, pp. 2143–2152, 2004.
- [251] A. Y. Wang, A. Ehrhardt, H. Xu, and M. A. Kay, "Adenovirus transduction is required for the correction of diabetes using Pdx-1 or neurogenin-3 in the liver," *Molecular Therapy*, vol. 15, no. 2, pp. 255–263, 2007.
- [252] L. J. Yang, "Liver stem cell-derived β -cell surrogates for treatment of type 1 diabetes," *Autoimmunity Reviews*, vol. 5, no. 6, pp. 409–413, 2006.
- [253] S. Gefen-Halevi, I. H. Rachmut, K. Molakandov et al., "NKX6.1 promotes PDX-1-induced liver to pancreatic β -cells reprogramming," *Cellular Reprogramming*, vol. 12, no. 6, pp. 655–664, 2010.

Review Article

Evolution of β -Cell Replacement Therapy in Diabetes Mellitus: Islet Cell Transplantation

Cyrus Jahansouz, Cameron Jahansouz, Sean C. Kumer, and Kenneth L. Brayman

School of Medicine, University of Virginia, Charlottesville, VA 22102, USA

Correspondence should be addressed to Cyrus Jahansouz, cj6h@virginia.edu

Received 29 May 2011; Accepted 8 August 2011

Academic Editor: Andrew Posselt

Copyright © 2011 Cyrus Jahansouz et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Diabetes mellitus remains one of the leading causes of morbidity and mortality worldwide. According to the Centers for Disease Control and Prevention, approximately 23.6 million people in the United States are affected. Of these individuals, 5 to 10% have been diagnosed with Type 1 diabetes mellitus (T1DM), an autoimmune disease. Although it often appears in childhood, T1DM may manifest at any age, leading to significant morbidity and decreased quality of life. Since the 1960s, the surgical treatment for diabetes mellitus has evolved to become a viable alternative to insulin administration, beginning with pancreatic transplantation. While islet cell transplantation has emerged as another potential alternative, its role in the treatment of T1DM remains to be solidified as research continues to establish it as a truly viable alternative for achieving insulin independence. In this paper, the historical evolution, procurement, current status, benefits, risks, and ongoing research of islet cell transplantation are explored.

1. Introduction

In part one of this two-part paper, pancreas transplantation was explored as the definitive treatment for patients with Type 1 diabetes mellitus (T1DM) [1]. It is estimated that of the 23.6 million people diagnosed with diabetes mellitus, 5–10% consist of patients with T1DM [2]. Moreover, recent reports indicate that the incidence of T1DM is increasing, with one study predicting an increase of 70% in those under the age of 15 by 2020 [3–6]. Accordingly, the significant population already afflicted with this disease compounded by the increasing incidence worldwide will have a tremendous impact on future healthcare both domestically and globally [7]. Estimates show that patients with T1DM treated with intensive medical management have six- to sevenfold higher direct cost than age-matched nondiabetics [8]. Although cost is a concern, it is the long-term complications of T1DM that result in the extensive morbidity in this population which fuel the desire for viable alternative treatments from the standard of care, intensive insulin therapy [9]. Even with the mainstay treatment, patients are still at significant risk for complications including retinopathy, neuropathy, nephropathy, coronary artery disease, peripheral vascular disease,

and cerebral vascular disease. While the etiology of this disease remains elusive, it is believed that a relationship exists between genetic susceptibility and environmental factors, including infections and toxins, which results in its fulminant presentation [10, 11].

The quest for a surgical treatment for T1DM first began more than a century ago with the likes of Oskar Minkowski and Josef von Mering at the University of Strasburg, Strasburg, Germany [12, 13]. It was not until 1966 when success was achieved by Kelly et al. who completed the first whole-organ pancreatic transplant at the University of Minnesota [14]. Soon thereafter, the concept of islet cell transplantation, originating with and developed by the visionary Paul Lacy and longtime research partner David Scharp at Washington University in St. Louis, would come into its research phases and be driven further with the likes of John Najarian and David Sutherland at the University of Minnesota [15–17]. Initially, it was met with tremendous optimism. However, the brilliant concept has been troublesome in allowing clinicians to maximize on the idealized potential that lies within it in treating patients with T1DM. Even now, the American Diabetes Association only endorses islet transplantation not

as a therapeutic alternative, but rather as “performed only within the setting of controlled research studies” [18].

This paper will now focus on islet cell transplantation as a potentially enhanced alternative therapy for intensive insulin therapy and as a minimally invasive alternative to pancreatic transplantation. It will begin with a brief history of islet cell transplantation, followed by its current state, and then the procedure’s benefits and risks. It will continue with a discussion of current research, highlighting barriers and potential therapies, to reduce islet mass loss following transplantation, and imaging as a means to follow the health of the islet mass. It will end with a discussion on islet autotransplantation as it stands today, primarily as an alternative for the treatment of chronic pancreatitis.

2. Brief History

Initially, the presence of the exocrine portion of the pancreas proved to be problematic in the transplantation of fragments of pancreas in animals due to the destructive nature of the enzymes [19]. However, this problem was circumvented in 1965 when Moskalewski used collagenase to separate intact islet from a guinea pig’s pancreas [19]. Islet cell transplantation subsequently was initiated by Ballinger and Lacy and Reckard et al., who, in 1972, were the first to report that isolated islets could reverse the effects of experimentally induced diabetes [20, 21]. Ballinger and Lacy transplanted 400 to 600 islets obtained from four donor rats intraperitoneally into their diabetic counterparts following the administration of streptozotocin (STZ) to induce the diabetes [20]. After islet cell transplantation, the recipient rats regained their normal weight, reduced their glycosuria, and achieved normoglycemia [20]. A key discovery in islet transplantation was when Kemp et al. compared graft efficacy as a function of graft location. They achieved normoglycemia in STZ-induced diabetic rats through injection of islet cells into the portal vein but not in rats in which islet cells were transplanted intraperitoneally [22]. Monkeys, however, proved to be more challenging. Scharp et al. were only able to partially alleviate STZ-induced diabetes in monkeys, which was attributed to an insufficiency of islet cells as well as allograft rejection [23].

Mirkovitch and Campiche made significant advances when they demonstrated that diabetic dogs could achieve normoglycemia by autotransplantation of pancreatic islet tissue [24]. Using collagenase to digest the pancreas, the partially purified islets were injected into the spleen through the splenic vein [24]. Subsequent splenectomy resulted in a diabetic state [24]. Kretschmer et al. demonstrated that direct injection of the pancreatic tissue into the splenic pulp was more effective than injection through the splenic vessels and the portal vein [25]. Mehigan et al. demonstrated the importance of the size of the minced particles and their influence on the outcome of islet transplantation in dogs. They also observed poor outcomes in relation to acinar cell atrophy and fibrosis from long-term ductal ligation [26, 27].

Yet, it was Sutherland et al. in 1974 who began the first human trials to treat diabetes using isolated islets from cadaveric donors [28]. Ten transplants were performed in seven

diabetic patients, all of whom had received a prior renal transplant for end-stage diabetic nephropathy. Although a reduction in the exogenous insulin requirement was observed, complete freedom from its use was not achieved. Failure of the grafts could not be attributed to any specific reason but rather secondary to a combination of rejection and inadequate islet cell mass [28]. In 1980, Largiader et al. became the first to report insulin independence following islet allotransplantation in a Type 1 diabetic [29]. The second report was not made until 1990 by Scharp et al. [30]. Socci et al., in a study of six islet-cell transplant recipients with T1DM, also achieved insulin independence in a patient who underwent islet after kidney transplantation. Six months following islet transplantation, the patient achieved insulin independence with normal values of HbA1c, 24-hr metabolic profile, and oral glucose tolerance test. This was sustained for a five-month period [31]. Other subsequent cases were reported throughout the 1990s from the Universities of Alberta, Minnesota, and Pittsburgh [32–34].

Between 1990 and 1995, 180 patients underwent islet cell transplantation worldwide [35]. Of these, 96 were recorded in the international islet transplant registry. 53% of the patients had islet cell function for as long as a week, but graft survival reduced to 26% after one year. Only 7% became insulin independent [35]. In 1994, the University of Giessen introduced protocol changes that significantly improved the efficacy of islet cell transplantation [36]. In all 12 of their patients, the islet graft survived for more than 3 months, and, in 9 patients, the graft functioned for at least a year. Four of these patients attained insulin independence [36, 37]. These results were confirmed independently with significant improvement in graft survival and insulin independence [38, 39].

Throughout the 1990s, and even through today, islet cell transplantation continues to face a number of challenges: transplanting an adequate mass of islets, the adverse effects of the diabetogenic immunosuppression, islet graft loss due to immunologic rejection, identifying an optimal location for transplantation, and overcoming the shortage of pancreata [40–42]. Arguably the most significant advancement in islet transplantation efficacy was made in 2000 by the Edmonton group, whose attempt to address the shortcomings of pancreas transplantation allowed for a tremendous improvement in the islet transplantation protocol. They transplanted an islet mass from two to four donors and avoided glucocorticoids while minimizing the use of calcineurin inhibitors. This was accomplished through use of sirolimus, low-dose tacrolimus, and daclizumab. As a result, they were able to achieve insulin independence in all the seven of their patients but required the use of 15 donor pancreases to do so [43]. In their follow-up international trial, 36 patients with T1DM received 77 islet infusions at nine sites. 16 patients (44%) achieved insulin independence at one year postfinal infusion with 10 patients maintaining partial graft function and the last ten with complete graft loss. These results, thus, confirmed the potential long-term viability and reproducibility of islet cell transplantation, albeit with room for achieving greater results [44].

3. Islet Isolation

Some of the most extensive research in islet transplantation has involved identifying avenues for improvement in the steps necessary to isolate an adequate islet cell mass. Following the procurement and preservation of the pancreas, islet cells undergo the following steps: digestion, purification, culture, assessment, and, lastly, transplantation.

According to CITR, 85% of reported islet transplants employed either the University of Wisconsin (UW) or two-layer methods for pancreas preservation [45]. The two-layer method (TLM), created by Kuroda et al., was developed as a means to increase oxygenation and protect organs from hypoxia through the use of perfluorocarbon during cold preservation [46, 47]. As a result of the oxygenation, adenosine triphosphate production is maintained at the perfluorocarbon and UW interface [47–49].

3.1. Digestion. One of the key advances in islet cell transplantation was the development of the automated method of pancreatic digestion with the use of the Ricordi Chamber, based on the work of Moskalewski and Lacy, which was able to increase islet yield [50–52]. Based on CITR, Liberase HI was the most commonly implemented collagenase, used for processing in 77% of cases, followed by Serva Collagenase NB1, used in 18% of cases [45]. Liberase HI was identified in the late 1990s as a collagenase that demonstrated superior enzymatic action over the traditional collagenase preparation (Type P) [53, 54]. However, concerns were raised regarding the small potential risk of bovine spongiform encephalopathy when it was revealed that Liberase HI is isolated from *Clostridium histolyticum* grown in media containing brain-heart infusion broth [55]. In Japan, three-year follow-up studies of recipients have revealed no incidences of prion diseases [56].

Concerns still remain; thus, research has been conducted for viable collagenase alternatives. Recently, Roche Diagnostics has provided the mammalian tissue-free Liberase MTF-S as an alternative to its Liberase HI [57]. In their study, Shimoda et al. compared four collagenases: Liberase HI, Liberase MTF C/T, Serva Collagenase NB1 Premium Grade, and Clzyme Collagenase HA. They indicated that the three alternative enzymes would enable for higher islet yields than with Liberase HI [58]. When comparing Collagenase XI to Liberase HI, Collagenase XI resulted in a decline in functional capacity of islets which was restored during cultivation. However, Liberase HI exhibited greater functional capacity during isolation and the subsequent seven days of cultivation [59]. O’Gorman et al. compared Liberase MTF to the Serva Collagenase NB1 and observed comparable results between the two collagenases [60]. Of note, in a large-scale comparison of Liberase HI to Collagenase NB1, Liberase was observed to be more efficient for pancreas dissociation but was observed to be more harmful to exocrine cells and islet tissue [61]. Szot et al. at the University of California, San Francisco, have also shown success by implementing the Serva enzyme blend of Collagenase NB1 and Neural Protease NB using a systematic approach and identifying donor criteria to achieve clinically implementable results [62]. Another

available collagenase, Vitacyte, was compared with Serva NB1 and not only showed comparable results but exhibited markedly decreased time required to release a significant islet number from acinar tissue, thus, potentially allowing for increased preservation of islet integrity in the future [63].

In summary, a number of enzymatic blends and collagenases are available, and more studies are being conducted not only as to their efficacy but also with regards to characterizing the microstructure which defines the pancreas to optimize its dissociation. Consequently, continued research is necessary to identify which product or mixture of components will result in the greatest islet yield and functionality.

3.2. Purification. After enzymatic digestion of the pancreas, the remaining contents then undergo purification to decrease transplanted tissue volume, albeit with minimal loss of islet cells. This step has typically been performed with the cell processor COBE 2991, which uses the differences in density of the islet cells and exocrine tissue to separate them [64–66]. The gradient media implemented in this step have traditionally been the Ficoll solution, first described by Lake et al. in the early 1970s, as another means to improve recovery of islet cells [65]. The process was further advanced by Olack et al., who used the organ preservation solution Euro-Collins to dissolve in which the Ficoll powder [47, 67].

According to CITR, all cases of islet transplantation implemented the use of a density medium [45]. While a number of density media have been researched, Iodixanol has recently revealed positive outcomes in islet yield [68, 69]. Noguchi et al. showed a much larger islet yield when using Iodixanol as compared to Ficoll solutions [68]. This may be attributed to its ability to reduce cytokine/chemokine production, which, as Mita et al. observed, led to a significant reduction in the loss of islet cells during culture [70, 71]. However, this group has observed comparable islet recovery rates to the Ficoll-based density gradient during density purification [70, 71].

3.3. Culture. As reported to CITR, 54% of islet masses were cultured, defined as six or more hours in a specially prepared nutrient medium, for a median time of 27 hours [45]. Generally, the most commonly used culture medium is the Connaught Medical Research Laboratory-based Miami-defined media no. 1, which has shown positive outcomes compared with alternative media [72, 73].

The use of culture has been somewhat controversial when compared with the use of fresh islets and has actually shown a reduction of islet mass and functionality [47, 74–76]. The University of Minnesota recently concluded that, while it is not disadvantageous with regards to recovery of islet function, there is increased expression of several stress-related genes [77]. Nevertheless, culturing islets has some advantages as it allows for functional assessment of islets, preservation during travel time, and recipient preparation in attaining therapeutic levels of immunosuppression [47, 78–80]. Immunologic advantages have also been observed [81, 82]. Furthermore, it may allow time for quality control and for modifications to promote islet survival [47, 80, 83].

In a study of 104 islet preparations, Kin et al. identified several factors by univariate analysis contributing to islet loss during culture, including longer cold ischemia time, two-layer method preservation, lower islet purity, and higher islet index. By multivariate analysis, they observed higher islet index and the use of the two-layer method as factors as well. Islet yield also significantly decreased after culture for 20 hours [84].

With regards to modifying the culture to increase islet yield, few potential supplements have emerged. Because of the relative impurity of cultured islets, the presence of exocrine tissue may be problematic in damaging the islet cells. Thus, Loganathan et al. recently showed improved islet recovery while preventing insulin cleavage with the addition of α 1-antitrypsin (A1AT) to culture, hypothesizing that the added enzyme may protect insulin from cleavage by protease activity [85]. Toso et al. described increased yield with the addition of liraglutide, the long-acting human glucagon-like peptide 1 analogue [86].

The temperature at which islets are cultured has also been a point of controversy. Most groups have based their culture at a range of 22–24°C based on the initial work by Lacy et al. [87]. Noguchi et al. recently observed improved outcomes of islet transplantation at 4°C (<5% loss) than that at both 22°C (19% loss) and 37°C (24% loss) [80]. Others have also observed decreased rates of recovery at 37°C relative to the lower temperatures as well [88, 89].

3.4. Assessment. While the quantification of islets remains a high priority, assessing their functionality prior to transplantation allows for a predictive component to the procedure to decrease the rate of posttransplant graft failure. The most widely used method is dithizone staining with manual and visual counting of islet equivalents under a light microscope, while viability has been evaluated by assessing membrane integrity with fluorescein diacetate/propidium iodide (FDA/PI) [90]. These methods currently have disadvantages which limit their usefulness. Major limitations include the assessment of three-dimensional islets in two-dimensional planes, lack of ability to identify irreversibly damaged plasma membranes that have not yet permeabilized, operator dependency, its inability to distinguish endocrine (islet) from exocrine (contaminant) tissue, and lack of correlation with mitochondrial function assays, nude mouse bioassay, and clinical outcomes [90]. As such, new methods have been and are currently being developed.

Computer-assisted digital image analysis has been gaining support as a means of providing more accurate, consistent, and reproducible results in quantifying islet cells. This was recently affirmed by a multicenter study involving all eight member institutions of the National Institutes of Health-supported Islet Cell Resources Consortium [91]. Others have validated this finding as well [92].

Measuring oxygen consumption rate (OCR) and OCR/DNA have gained attention in their ability to predict islet graft function and diabetes reversal [93–96]. Papas et al. applied the measurements in a model for predicting transplant outcome in mice and obtained sensitivity and

specificity values of 93% and 94%, respectively. The measurements were also found to be valuable in predicting the marginal mass required for reversing diabetes [97]. Sweet et al. have demonstrated that the glucose-stimulated changes in OCR were predictive of diabetes reversal in mice and that the changes observed were more due to islet cells than nonislet cells [94, 95].

With regards to viability, Papas et al. compared the ratio of ATP to DNA with the ratio of ADP to ATP. They discovered that ATP/DNA as a better measure of viability as ATP levels fluctuate significantly and reversibly with metabolic stress [96]. They also cited the substantial disagreement that exists as to the significance of the ADP/ATP as well as a reason for its limited use [98–100].

The University of Wisconsin recently presented a multiparametric objective approach to assess islet quality based on mitochondrial membrane potential (MMP), *in vitro* glucose-stimulated insulin secretion (GSIS), and ATP to ADP ratio as a marker of reduced oxidative phosphorylation and achieved an accuracy of more than 86% in predicting *in vivo* functional potency [101].

4. Current State and Statistics

Of the 46 islet transplant centers polled in North America by the Collaborative Islet Transplant Registry (CITR) from 1999 through 2008, 32 centers performed at least one islet allograft transplant, with 27 of those centers reporting detailed information to the registry [45]. From that time period, the CITR report includes 81% of both human islet allograft recipients and procedures conducted in North America. It includes 412 recipients of islet transplants receiving 828 infusions from 905 donors. Of the 412 recipients, 347 (84%) received islet-alone (IA) infusions while 65 recipients (16%) had received a kidney transplant prior to receiving islet infusions (IAK: Islet After Kidney). Both mean recipient and donor age was 44 years, with recipients characterized by a mean duration of diabetes of 28 years. Mean time from cross-clamp to pancreas recovery was 44 minutes, while cold ischemic time was 7.3 hours. Only 11–15% of patients remained insulin-independent throughout the first year [45].

Figure 1 illustrates the total number of islet transplant procedure performed and the number of recipients in the 32 active North American transplant centers [45]. Figures 2 and 3 illustrate possible states after first and last infusion [45]. In 2008, 66 islet allograft procedures were performed, with 32 patients receiving their first allograft. Both are an increase from 2007, in which 42 procedures were performed with 20 patients receiving their first allograft. However, these are still only approximately half the number of procedures performed and patients receiving a first allograft compared to 2005 [45, 102].

By the end of the first year following islet infusion, 65% of IA patients were reinfused. 8–12% of IA recipients retain detectable C-peptide while being insulin dependent. Without reinfusion as a factor, insulin independence in IA recipients declines to 27% at year 3. Furthermore, from the last infusion, the rate of loss of islet function steadily increases from

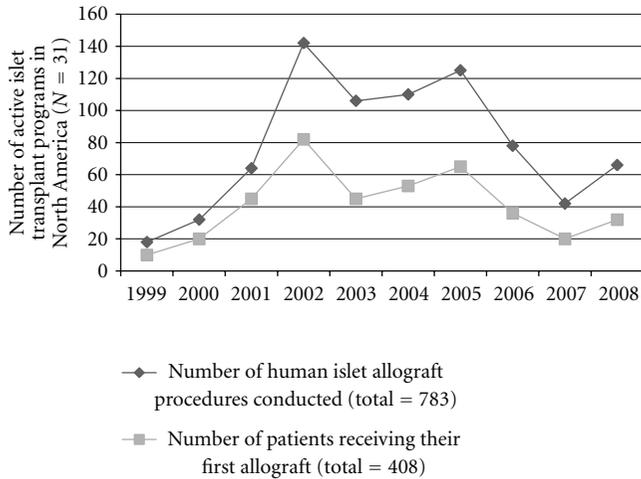


FIGURE 1: Total number of islet transplant recipients and total infusions in North America, 1999–2008, based on the CITR 2009 Annual Report [45].

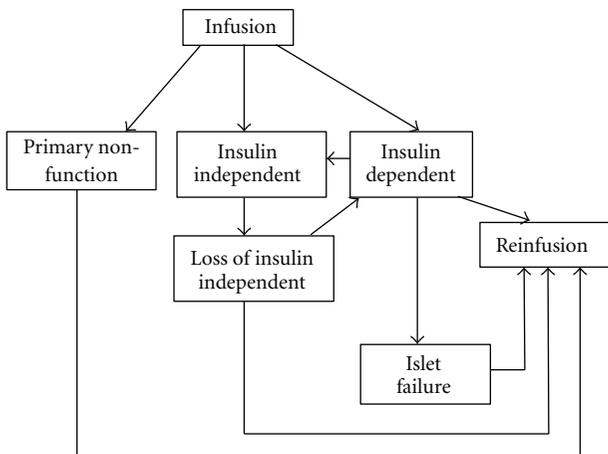


FIGURE 2: Possible states after first infusion.

12% at month 6 to 42% at year 4. The proportion of patients retaining graft function with exogenous insulin over the three-year period remains in the range of 19–31%. Similar to IA recipients, postfirst infusion rates for IAK remain near 20%. Postlast infusion rates remain consistently below those of IA recipients through the four-year period as well. These trends of increasing graft loss and decreasing insulin independence over time following infusion prevail regardless of the total number of infusions given, although these rates differ somewhat [45].

If the number of infusions is taken into consideration, a second- or third-repeat infusion has a more significant role in increasing the proportion of insulin-independent recipients from the beginning of infusion to the 500-day period after infusion. Thus, the greater number of infusions a patient receives, the quicker the recipient will attain insulin independence. Thereafter, the percentage of insulin-independent patients declines at a similar rate until the 900 day mark to the 1100-day mark (3-year mark) regardless of

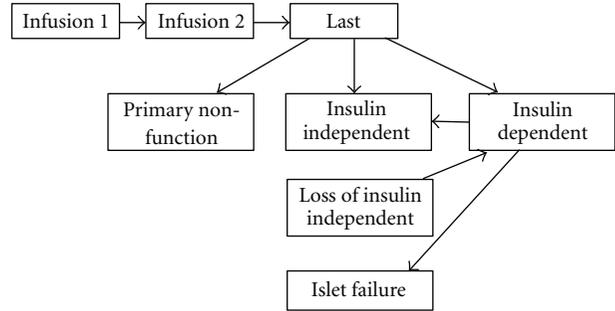


FIGURE 3: Possible states after last infusion.

the number of infusions. Of the patients who attain insulin independence, 70% maintain this status after one year, and 45% maintain it at three years. Furthermore, graft function continues to decrease over time as well, with 35% of all recipients losing graft function at the three-year period after their last infusion [45].

At this point, it may be logical to compare the results of islet cell transplantation that of pancreas transplantation. However, a true comparison with regards to graft function at this time cannot be performed due to the far superior results of pancreas transplantation. Following pancreas transplantation, 1-year posttransplant graft survival remains ~78–85% and at 3 years, 60–80%. Patient survival in pancreas transplantation at 1 year exceeds 95% for all three categories: Simultaneous Pancreas Kidney (SPK), Pancreas After Kidney (PAK), and Pancreas Transplant Alone (PTA) [103–105]. 3-year survival rates exceed 90%. Speight et al. did perform a review of twelve studies which compared patient-reported outcomes (PRO) of Pancreas After Kidney (PAK), Pancreas Transplant Alone (PTA), IAK, and IA transplantations, and found benefits with regards to fear of hypoglycemia, diabetes-specific quality of life, and general health status. On the other hand, shortcomings were observed with short-term pain, immunosuppressant side effects, and depressed mood associated with loss of graft function. Thus, as they concluded, much has yet to be learned in terms of patient-based quality-of-life outcomes in comparing the different types of pancreas transplantation with islet cell transplantation [106].

Optimistically; however, while there is no standard tool to effectively monitor islet cell rejection, Toso et al. monitored the immune reactivity against islet cell grafts in mice using enzyme-linked immunosorbent spot (ELISPOT) assay to identify the ex vivo release of γ IFN from splenocytes stimulated by islet donor extracts. They were able to demonstrate transiently increased levels of immune reactivity, as indicated by reactivity of splenocytes against islet proteins, in allogeneic models, and were able to achieve a sensitivity of 70% and specificity of 94% [107]. In the future, such data, combined with the gradual improvement in islet efficacy, may prove to influence and help guide the patient’s decision as to the appropriate treatment thereafter.

5. Benefits

Islet cell transplantation has been endorsed as having a largely beneficial impact by several groups with regards

to achieving stronger metabolic control over brittle diabetes and reducing the tremendous physiologic impact of T1DM. Although improvements in the counterregulation and symptom-recognition mechanisms with respect to glucagon and epinephrine may be observed, values continue to be considerably below normal [108–110]. However, this drawback may be mitigated as growth hormone levels are restored and normalized [107]. Furthermore, autonomic and neuroglucopenic hypoglycemia warning symptoms return even in individuals with longstanding diabetes [111]. Long-term benefits of islet graft function include near-normal HbA_{1c} levels and reasonable glucose control with occasional insulin independence [112]. According to CITR, the percentage of IA recipients with normal HbA_{1c} levels increased from 2% preinfusion to 51–60% at year one after last infusion [45]. In a study of seven IAK transplant recipients, a near two-point reduction in HbA_{1c} was observed with 30% achieving 1 year insulin independence and 86% with one year graft function. No severe hypoglycemic events were reported [113]. In their study, Warnock et al. enrolled 10 patients with diabetes-induced renal dysfunction in a best medical therapy program and then crossed them over to islet transplantation. All patients showed improved metabolic control reducing HbA_{1c} from a mean of 6.9% after best care to 6.2% 6 months after islet transplantation [114]. Poggioli et al. observed significant nutritional and dietary changes in 30 of 52 islet transplant recipients, including substantial reductions in body weight, body mass index, waist circumference, and fat weight [115]. Considerable progression of diabetic retinopathy is also much more likely in patients with intensive insulin therapy as opposed to islet transplant recipients, in whom it was shown to stabilize [116, 117].

Cardiovascular function improved as well in patients with end-stage renal disease (ESRD) receiving both kidney and islet transplants relative to patients receiving kidney only, with improvements in atherothrombotic profile and endothelial morphology [118]. The same IAK group also had improvements in ejection fraction and peak end-diastolic volume (EDV) and stabilization in time to peak filling rate. These indices were diminished in the kidney-only group [119]. Furthermore, renal graft survival and function were also improved when combined with islet cell transplantation [120]. Poor long-term outcomes of polyneuropathy were also prevented in patients undergoing the IAK procedure, as evident with a reduction in advanced glycation end products (AGEs) and expression of their specific receptors (RAGE) [121]. Lee et al. have also shown that patients may stabilize or even demonstrate improvement of their diabetic neuropathy [117].

6. Risks

In the short term, the risk associated with islet transplantation is pointedly less, in comparison with whole-organ pancreatic transplantation. However, similar to pancreas transplantation, longer-term complications are likely associated with the chronic necessity for immunosuppression and are highlighted by the well-known calcineurin inhibitor-induced

nephrotoxicity, which becomes more important due to the potential preexistence of diabetic nephropathy [122–125].

The Edmonton Group, in a review of 34 patients undergoing 68 procedures, recorded potentially serious complications in only 6 of 68 procedures [126]. Complications included two patients with portal venous thrombosis and four patients with clinically significant intra-abdominal hemorrhage [126]. Bleeding was also observed in 18 of 132 percutaneous transhepatic islet transplants in 67 patients by Villiger et al. from 1999 to 2005. However, they did conclude that the complication is avoidable if the intraparenchymal liver tract is sealed effectively [127]. Maleux et al. reported on 15 patients who underwent 31 procedures. Only three patients presented with complaints of transient abdominal pain, which furthered the notion that percutaneous transhepatic injection of islet cell grafts is a safe and reproducible procedure [128]. From 1992 through 2003 at the University of Geneva Hospital in Switzerland, 62 percutaneous transhepatic injections were performed. Nine complications (14.5%) were observed, of which two were portal vein thrombosis and seven were intra-abdominal hemorrhage [129]. In their study of seven IAK recipients, Cure et al. reported two procedure-related pleural effusions and one episode of cholecystitis, all of which resolved [113].

As mentioned above, sensitization is another potential threat following a failed islet transplant. This was illustrated by the Edmonton Group in which 16% of the recipients became sensitized after transplantation, with *de novo* antibodies seen in 36% of sensitized and 33% of nonsensitized recipients [130]. In their international trial, they reported procedural-related complications including acute intraperitoneal bleeding in 7 of 77 (9%) with four requiring blood transfusions and the other requiring laparotomy. No cases of portal vein thrombosis were reported. Two of the 36 patients had partial portal branch vein occlusions, but they were successfully treated with anticoagulation [44].

7. Ongoing Debate and Limitations

Central to the debate has been whether insulin independence should be the main objective in islet cell transplantation or whether it should simply be to achieve acute and long-term metabolic control and to improve the quality of life of individuals with brittle diabetes [131]. At the present time, support lends itself to the latter. Once again, the international trial of the Edmonton protocol concluded that, even with normal endocrine reserves rarely being achieved, insulin independence is gradually diminished over time. Considerable metabolic control, however, is achieved with protection from hypoglycemia and improved HbA_{1c} levels, thus, favoring the procedure for highly selected patients after exhausting all other therapeutic options [44]. Recently, the GRAGIL group released similar results favoring the use of islet transplantation as a therapeutic means to achieve stronger metabolic control with respect to restoring beta-cell function rather than measuring success by the achievement of insulin independence [132].

One observed limitation of islet cell transplantation is the great variation in achieving insulin independence from

center to center worldwide, which is primarily attributed to a lack of experience [43]. Another study by the GRAGIL Consortium proposed a solution to bypass this problem by being the first to employ the Edmonton protocol in a multicenter setting [133]. They have demonstrated further feasibility of the multicenter approach by illustrating the absence of ill effects with regards to shipment of islet cells, discussed below [134].

To combat the limitations associated with islet cell transplantation, it may be best to steer the therapy towards those patients with the most potential for graft survival and who may benefit the most: high-risk patients with recurrent episodes of hypoglycemia [135]. Two of the most important and recurrently identified aspects regulating islet survival have been auto- and alloimmunity and the maintenance of a sufficient islet cell mass. At the Leiden University, it was shown that the presence and amount of autoimmunity to one or two antigens determine the survival of islet grafts and, as such, imply a role in patient selection in the future to maximize graft efficacy and adjust graft size as needed [136]. Ironically, it has recently been shown that the immunosuppression regimen implemented by the Edmonton protocol may exacerbate this autoimmunity. Monti et al. recently reported that the protocol may actually be causative in the long-term failure of islet cell transplantation. Employing the protocol often results in lymphopenia that is associated with elevated serum levels of the homeostatic cytokines IL-7 and IL-15, which expands the autoreactive CD8⁺ T-cell population [137].

7.1. Problems with Immunosuppression. As has been documented, many of the immunosuppressants required in islet transplantation have also been shown to adversely affect the transplanted islets. One such familiar consequence of the use of corticosteroids is hyperglycemia as a result of insulin resistance occurring from the reduction of insulin-mediated glucose uptake and utilization [138]. Both Sirolimus and Tacrolimus inhibit beta-cell regeneration and prevent the normalization of glucose homeostasis in treating diabetic mice as well [139]. Tacrolimus has also been observed to decrease insulin gene transcription, the stability of insulin mRNA, in vitro insulin synthesis and mitochondrial density, and in vivo insulin secretion, while Sirolimus decreases in vitro insulin synthesis and secretion, ductal cell regeneration, and angiogenesis [140–149]. Additionally, mycophenolate mofetil is a potent inhibitor of ductal neogenesis and has been shown to impair glucose-stimulated insulin secretion [150, 151]. However, Johnson et al. have shown that, to some extent, these negative effects may be counteracted with the glucagon-like peptide-1 exenatide. The use of exenatide has shown positive effects on the islet cell graft in stimulating insulin secretion and improving graft function, thus, aiding in glycemic control [151–157].

While a variety of groups have been able to achieve insulin independence with single donor islet transplantations, the protocol for this achievement still varies from group to group [158]. The University of Minnesota achieved insulin independence in all the eight of its patients with each patient receiving only one islet graft. Their protocol consisted

of daclizumab, etanercept, and thymoglobulin for induction, with mycophenolate mofetil, Sirolimus, and either no or low-dose Tacrolimus. Five of eight patients maintained insulin independence beyond one year, and, in the three patients who experienced graft failure, it was preceded by subtherapeutic Sirolimus exposure without measurable Tacrolimus trough levels [78]. Improved longer-term outcomes have been achieved at the University of Minnesota as well. Six patients underwent one or two islet graft infusions with a protocol of thymoglobulin for induction along with etanercept, cyclosporine, and everolimus for maintenance for the first year following transplantation. Thereafter, mycophenolate mofetil or mycophenolic acid substituted for everolimus. Five patients were insulin independent at one year, while four remained so at 3.4 ± 0.4 years after transplant [159].

At the Emory University, they compared the Edmonton protocol, highlighted earlier, with a protocol consisting of daclizumab induction, a 6-month course of Tacrolimus, and maintenance with efalizumab and mycophenolate mofetil. While two patients achieved insulin independence in the Edmonton protocol, all four patients with the novel protocol did so [160]. In another study, Matsumoto et al. compared two common immunosuppression protocols on six patients: in the first, three patients were placed on daclizumab for induction, with Sirolimus and Tacrolimus for maintenance along with etanercept as an anti-inflammatory agent; while in the other, three patients were not only placed on thymoglobulin for induction and tacrolimus and mycophenolate mofetil for maintenance along with anakinra (anti-IL- β) and etanercept but also provided islet cells with iodixanol purification. While all patients became insulin independent, the former protocol required two infusions to do so [161].

At the University of California, San Francisco, a group of ten patients with T1DM underwent islet transplantation and was treated with a protocol of thymoglobulin induction and maintenance with Sirolimus or mycophenolate and either belatacept (BELA) or efalizumab (EFA). While EFA is no longer available for clinical use, all five patients who received BELA achieved insulin independence after a single islet graft, with only one requiring insulin use 305 days following transplantation [162].

With increasing knowledge of the negative effects of certain immunosuppressants on β -cell function, it is hopeful that novel protocols will continue to develop, and ones that have achieved success in smaller populations will be implemented on a larger scale so that standardized protocols may be established. Thus, one may be optimistic that improved protocols may lead to stronger results in the near future.

7.2. Inflammation, the Immune Response, and Oxidative Stress. Perhaps the major barrier in islet transplantation is the inevitable decline of islet graft function over the short and the long terms. Shortly after intraportal transplantation, more than 60% of islet cells undergo apoptosis during the revascularization period [163–165]. During the engraftment process, which may last up to two weeks, oxygen is received primarily through passive diffusion, thus, creating an environment of oxidative stress [166, 167]. This hypoxic state is

an important contributor to islet dysfunction with resultant apoptosis and necrosis [168, 169]. One of many facets of this hypoxic injury is the role that inducible nitric oxide synthase (iNOS)-nitric oxide (NO) has in signaling apoptosis [170, 171]. Another is the hypoxia-induced activation of AMP-activated protein kinase in cytokine-induced apoptosis [172–174]. Subsequently, increased metabolic demand is required of the remaining islets, which may lead to metabolic exhaustion and dysfunction [175].

Defenses, consisting of the innate and adaptive immune responses, also contribute to the substantial islet cell loss [176]. The innate immune system creates an environment ill suited for the survival of the sensitive islet cells. Conversely, the adaptive immune response is better controlled with current immunosuppressive protocols [176, 177]. Not surprisingly, cytokines and low-grade systemic inflammation promote islet cell dysfunction and death as well [178–181]. One such cytokine is Tumor Necrosis Factor (TNF) α and its known toxicity to islet cells [180, 181]. Thus, as noted above, the implementation of etanercept has been more widely implemented in recent studies at multiple institutions with promising results [78, 159, 161]. It is hopeful that these results will be observed once again in the multicenter Phase 3 trial implementing the Clinical Islet Transplantation Protocol 07 currently being conducted by the Clinical Islet Transplantation Consortium [182]. The inflammatory environment in which islets are placed continue to form a central barrier to successful graft survival, and targeting it at different levels may achieve more successful results [177].

A number of studies have shown that an avenue of improving islet viability may lie with the role that Toll-like receptor (TLR) activation has in mediating early islet graft failure. As part of innate immunity, it activates pathways such as NF κ B, and, if it is inhibited or even partially suppressed, it may aid in the grafting process [183–185]. Goldberg et al. have shown that carbon monoxide exposure to isolated donor islets may in fact provide some protection by blocking the TLR upregulation that occurs during the isolation procedure [186]. Following activation of the transcription factor NF κ B pathway, there is upregulation of genes mediating inflammation and apoptosis, thus, supporting its role as one of the mechanisms of islet loss as well as its blockade as a potential therapy [187–192]. An additional potential target may lie with the high-mobility group box 1 (HMGB1) due to its role in mediating early graft loss by stimulating hepatic mononuclear cells, upregulating CD40 expression, and enhancing IL-12 production by dendritic cells [184, 193].

An alternative avenue of immunologic research lies within the role of chemokines, notably monocyte chemoattractant protein-1 (MCP-1)/CCL2, constitutively expressed in islet cells and their role in monocyte recruitment, insulinitis, islet engraftment, and graft destruction [194–198]. Ogliari et al. have shown that higher donor levels of MCP-1/CCL2, as seen with brain death, lead to decreased graft survival in SPK recipients, likely further contributing to the posttransplant inflammatory state [199]. Similarly, Saito et al. observed a high expression of both tissue factor and MCP-1/CCL2 expression in isolated islets resulting from brain death and

ischemic stress in the rodent model, thus, emphasizing a role for pancreatic management from brain-dead donors [200]. Melzi et al. have suggested that strategies to decrease recipient MCP-1/CCL2 may be more fruitful [201]. Lee et al. indicated beneficial results in the mouse model when blocking MCP-1/CCL2 binding to its receptor, CCR2 [202]. Interestingly, two key mediators in the chemokine's release are NF κ B and Angiotensin II, which is actively generated in the pancreas, through their increased expression of MCP-1/CCL2 mRNA and protein [203, 204].

Correspondingly, a few adjunctive therapies have shown potential in improving islet survival. A role may exist for adenosine A(2A) agonists as they improve glucose-stimulated insulin secretion and inhibit inflammatory islet damage in the peritransplant period [205, 206]. Intensive insulin and heparin administration have also shown benefit in the peritransplant period [207]. Heparin's beneficial effects likely stem from its favorable impact against the instant blood-mediated inflammatory reaction (IMBIR) [83, 207].

While research continues to illustrate the barriers that exist in the peritransplant period, several potential therapeutic targets have been characterized, with a few therapies showing benefit. Work still remains in this phase and will only continue to shed light on the tremendous immunological underworks that characterize this crucial time frame of islet stress.

7.3. Optimal Location. A critical facet of islet cell transplantation remains the optimal site of implantation. As noted above, Kemp et al. were the first to demonstrate success with intrahepatic transplantation and thus has remained as a favored site for some time [22]. Glucagon unresponsiveness to hypoglycemia remains a consideration, as noted earlier, which is in contrast to that seen in whole-organ pancreatic transplantation. This is thought to be due to the increased intrahepatic glucose flux masking systemic hypoglycemia [208–213]. Liver ischemia and procedure-related complications, such as hemorrhage and thrombosis, are also concerns [214–217].

Accordingly, several locations have been considered as possibilities for the future, including vascular (celiac artery, spleen, lung), organ (renal subcapsule, pancreas, intramuscular, omental pouch, intraperitoneal, subcutaneous), and immunoprivileged (intracisterna magna, testis, and thymus) sites [217]. Recently, Kim et al. compared the kidney, liver, muscle, and omentum as islet transplant sites, evaluating each based on operative feasibility, implantation efficiency assessed as marginal mass required and mean time to achieve normoglycemia, and glycemic control in the mouse model [218]. They observed that the omentum may be an optimum site in terms of implantation and efficiency, albeit with disadvantages. Namely, it does not allow repeat transplantation, and it is not possible in patients with a past laparotomy. On the other hand, muscle offers ease of operative feasibility but less vascularity. While the liver resulted in much greater mortality and delayed graft function, it afforded greater marginal mass. Interestingly, the kidney produced excellent

results in feasibility, efficiency, and glycemic control, but, as noted, differences exist with respect to the human kidney, as the subcapsule allows for less elasticity and affords limited space [218, 219]. Recently, the femur bone marrow cavity has also been introduced as a potential site of transplantation of a bioartificial pancreas (BAP), as reported by Yang et al. The BAP was composed of mouse insulinoma cells encapsulated in agarose gel further enclosed in a calcium phosphate chamber [220]. The group also evaluated the possibility of applying the BAP to intramuscular space in a comparison with the intramedullary cavity but reported increased effectiveness with the latter [221].

Further research has recently been performed with regards to an intramuscular transplant site. Directly comparing the intraportal site to muscle (biceps femoris) in rats, Lund et al. observed twice the necessary IEQ to achieve normoglycemia in muscle [222]. Others have shown some feasibility of intramuscular implantation as well, observing much better oxygenation when compared to the renal subcapsular site in rats but naturally less oxygenation than that in native pancreatic islets of nontransplanted controls [223]. Of note, Christoffersson et al. showed the importance of neutrophils in restoring inra-islet perfusion following transplantation at an intramuscular site [224, 225].

Performed in a variety of ways, several reports have been published with regards to increasing the vascularization of islet cells transplanted intramuscularly or subcutaneously. As will be discussed later, Witkowski et al. achieved excellent results when pretreating intramuscular sites with a biocompatible angiogenic scaffold before transplantation [226]. Salvay et al. created microporous polymer scaffolds produced from copolymers of lactide and glycolide, which were then adsorbed with collagen IV, fibronectin, laminin-332, or serum proteins before being seeded with 125 mouse islets. The scaffolds were then implanted onto the epididymal fat pad in mice. The scaffold with collagen IV maximally enhanced graft function promoting graft efficacy [227].

A number of studies have shown the potential clinical impact adjunctive treatment with VEGF has on increasing islet graft efficacy and viability [228–232]. Stiegler et al. used a combination of foam dressing, vacuum-assisted wound closure, and hyperbaric oxygenation (HBO) in rats, with results indicating increased vessel ingrowth and vascular endothelial growth factor (VEGF) levels dependent on duration of HBO treatment. Perfusion was significantly improved in the experimental group with only a small amount of apoptosis following transplantation [233]. Similarly, islet cells transplanted subcutaneously with adipose tissue-derived stromal cells (ADSCs), and minced adipose tissue showed increased vascularization and higher capillary density than mice implanted with either ADSCs or minced adipose tissue alone [234]. Ohmura et al. indicated that ADSCs promote survival and insulin function of the graft and reduced the islet mass required for reversal of diabetes [235]. Ito et al. demonstrated improved islet graft function and promotion of graft revascularization when islet cells were cotransplanted with bone marrow-derived mesenchymal stem cells in rats [236]. Duprez et al. were able to create composite cells of mesenchymal stem cells and islet cells, and

they showed beneficial results with regards to minimizing the immune reaction with blood and suppressing lymphocyte proliferation [237]. Other groups have observed similar immunosuppressive results and improvements in vascularization with mesenchymal stem cells as well [238, 239].

In another novel study, Shimoda et al. used an ultrasound-mediated gene-transfer method named ultrasound-targeted microbubble destruction (UTMD) to deliver non-viral plasmid vectors encoding VEGF into the host liver of mice. They observed that the VEGF gene promoted islet revascularization following transplantation and improved rates of achieving normoglycemia [240]. Similarly, Kheradmand et al. created an innovative approach to transplant islets through a combination of mechanisms. They created an extrahepatic site by transplanting islet-loaded microporous poly(lactide-co-glycolide) (PLG) scaffolds into the epididymal fat pad in mice. Ethylcarbodiimide- (ECDI-) treated splenocytes were infused as a tolerance induction strategy. Altogether, they experienced excellent results superior to intraportal transplanted islets [241]. Vaithilingam et al. observed increased levels of hypoxia-inducible factor-1 α (HIF-1 α) and VEGF expression when transplanting encapsulated human islets pretreated with desferrioxamine (DFO) into the peritoneal cavity of mice [242]. Others have noted the use of DFO to stimulate VEGF expression and islet vascularization as well [243–245].

While the intrahepatic site is the classic location for islet transplantation, it is likely that other sites will take over this role. A number of novel approaches have been discussed, and, considering the improvements observed with inducing vascularization, an intramuscular location may prevail as the leading candidate to replace the liver.

7.4. Shortage of Supply. Another major hindrance to human islet cell research and transplantation remains the shortage of pancreata. Potential solutions to increase resources lie in stem cells and xenotransplantation, both of which are being extensively researched, and in international islet shipping. At this point, we will focus on recent studies involving the shipment of islet cells. Vaithilingam et al. recently demonstrated success of shipping encapsulated islets from Chicago, Ill, to Sydney, Australia, achieving a recovery rate of 88%. Islets were encapsulated with a barium alginate microcapsule and were isolated for a median total of 11 days before being transplanted in mice [246]. Similarly, Qi et al. showed success with long-distance shipping of encapsulated alginate calcium/barium microbeads, maintaining *in vitro* and *in vivo* islet function [247]. Other groups have established the improved survival and functionality of alginate-encapsulated islets as well [248–250]. Ikemoto et al. have also had some success, shipping islets from Dallas, Tex, to Fukuoka, Japan. Islets were packed in either gas-permeable bags or non-gas-permeable bags. Recovery rate was higher in the gas-permeable group than the nongas-permeable group: $56.4 \pm 10.1\%$ versus $20.5 \pm 20.6\%$, $P < 0.01$. Purity also decreased to a greater extent in the nongas-permeable group [251]. Ichii et al. have endorsed the use of gas-permeable bags as well and have promoted shipments following cultured islets as opposed to that immediately after isolation [252].

Because of the changes in pressure and temperature islets must endure during shipment, Rozak et al. have suggested the use of containers equipped with commercially available TCP Phase 22 phase change material (TCP) and custom-designed pressure regulated gyroscopic shipping containers (PRGSC), which illustrated excellent environmental control by limiting temperature and pressure changes [253].

8. Further Research

Optimistic findings with regards to genetic manipulation have been observed, with caspase inhibition showing promise. Islet cells transduced with an X-linked inhibitor of the apoptosis protein (XIAP) expressing recombinant adenovirus were resistant to apoptosis. By inhibiting caspases 3, 7, and 9, this reduced the required transplanted islet cell mass [254–256]. However, a drawback remains the required use of adenoviral gene therapy.

Expanding on caspase inhibition, Emamaullee et al. employed a short course of the caspase inhibitor zVAD-FMK and demonstrated efficacy in enhancing marginal mass post-transplant grafting. Consequently, this illustrated the extent of damage caused to the islet implants by ischemia. zVAD-FMK selectively inhibits caspases 1–10 and 12. With renal subcapsular islet infusion, 90% of zVAD-FMK-treated mice became euglycemic with 250 islets versus 27% of the control animals. With portal infusion, 75% of zVAD-FMK-treated animals established euglycemia with only 500 islets, and all of the controls remained severely diabetic. No systemic toxicity was demonstrated [257].

In another study, Emamaullee et al. utilized another caspase inhibitor, EP1013 (zVD-FMK), which selectively inhibits caspases 1, 3, 6, 7, 8, and 9, as opposed to the less specific zVAD-FMK. No discernable difference was observed between the two caspase inhibitors with islets injected in the subcapsular space, but there was a significant difference observed with islets transplanted intraportally. Nearly 100% of the EP1013-treated animals achieved euglycemia with 500 islets, while only 62.5% of zVAD-treated animals, and 0% of the controls established euglycemia. Once again, no systemic toxicity was observed [258].

More recently, the group conducted another study to observe the combined effects of EP1013 with CTLA4-Ig, a costimulatory blocking agent shown to be an effective immunomodulatory agent [259]. Fully major histocompatibility complex (MHC) mismatched mice underwent islet allotransplantation. 40% of mice which were administered CTLA4-Ig alone resulted in prolonged islet survival of greater than 180 days, whereas 91% of mice administered both EP1013 and CTLA4-Ig showed prolonged survival. Treatment with EP1013 alone did not result in prolongation of allograft survival. Furthermore, in the study, they showed that the complimentary effects of both drugs reduced the frequency of intragraft CD4+ and CD8+ T cells both at short and long terms, and reduced the functional alloreactive T cell response along with B-cell allosensitization [260]. Thus, there is reason for optimism that this type of therapy could dramatically reduce the number of islets required to induce

insulin independence, reduce early immune stimulation from dying islets, and improve current immunosuppressive regimens to decrease or rid of the need for nephrotoxic agents.

At the University of Pittsburgh, growth factors, such as hepatocyte growth factor (HGF), and signaling molecules, such as protein kinase B (PKB)/Akt, have also shown promise [261]. Furthermore, combination gene therapy may have a role in posttransplant therapy, as shown by the co-expression of VEGF and interleukin-1 receptor antagonist and its resultant success in islet survival [262].

9. Imaging

Central to monitoring the progression of islet cells following transplantation is the role noninvasive imaging will have in the future, and, to this end, magnetic resonance imaging (MRI) is one of the major imaging modalities which may prove valuable. In 2004, Jiráček et al. was the first to report a technique for *in vitro* labeling of isolated pancreatic islets with the MR-contrast agent Ferucarbotran, composed of crystalline iron nanoparticles with superparamagnetic properties coated by carboxydextran, allowing for increased hydrophilicity and increased uptake by cells [263, 264]. Ferucarbotran is uptaken by islet cells by means of endocytosis without subsequent deleterious effects on function [265–267]. The feasibility and safety of this model, specifically iron labeling, to humans was then demonstrated by Toso et al. in four patients receiving a total of nine islet transplants [267]. Its safety was further noted by Kim et al. who observed no deleterious effects on either islet function or gene expression [268].

The first clinical human trial implementing this imaging modality was performed recently by Saudek et al. in eight patients with T1DM [269]. No side effects related to the modality were observed. With regards to efficacy in observing pancreatic mass on MRI, they noted that the labeling period was less effective if islets were incubated with Ferucarbotran for less than 16 hours. Decrease in visualization occurred one week following transplantation, thus, correlating with the oft observed early destruction of islets. This also corresponds with a previous study in rats [270]. Thereafter, visualization remained stable for up to 24 weeks. As they concluded, while the modality allows for precise localization and quantification, an exact correlation between total number of transplanted islets and hypointense spots (as observed on MR) should not be expected due to a number of suspected factors including islet cell destruction, islets seeding together, lack of detection in counting, and random decreased contrast uptake.

While these studies are in their early phases for clinical application, they represent important steps towards enhancing the monitoring of islet cell transplantation [269]. Recently, ferumoxide has also been introduced as a labeling agent. Though it exhibits a similar safety profile to Ferucarbotran, it exhibited inferior iron uptake by islet cells and increased hepatic clearance, thus, affording less background [271–274]. In a distinctive use of the MRI, manganese-enhanced MRI has also been used to successfully quantify

β -cell mass in both static and dynamic conditions without manganese-associated toxicity, otherwise characterized by changes in insulin production. While still in its research phase of implementation, this may yet serve as another potential avenue for further research of islet graft monitoring [275].

In addition to MRI, bioluminescent imaging has also been a source of optimism for its potential use in posttransplant islet monitoring [276–281]. First performed by Lu et al., the group transduced isolated human and rodent islets with recombinant adenovirus or lentivirus vectors expressing a firefly luciferase gene under the control of the nonspecific cytomegalovirus promoter. The promoter is not subject to regulation by blood glucose levels so as to accurately reflect the remaining islet graft mass [276]. Luciferase, when it reacts with its substrate, D-luciferin produces a photon emission that may be detected by a cooled charge-coupled device (CCD) camera. Following implantation, they discovered that the CCD signal was proportional to the implanted islet graft mass and that the lentivirus-engineered islets could be repetitively imaged long term after transplantation [276]. In a follow-up study, Chen et al. implemented the bioluminescent imaging model to determine how a change in functional islet mass correlated with metabolic abnormalities during the course of posttransplant rejection. They found that imaging modality was very sensitive, with bioluminescent signals observed from as few as 10 islets implanted in a variety of locations. Intensity stabilization occurred within two weeks and remained so for as long as 18 months after transplant [277]. Virostko et al. and Grossman et al. also implemented the bioluminescence model with transgenic mice expressing luciferase and obtained similar results, noting that small changes in recovery of bioluminescence correlated with major changes in blood glucose control [279–281].

As mentioned earlier, in another novel technique, Witkowski et al. examined an intramuscular transplantation site and followed graft progression using positron emission tomography (PET) imaging with [¹¹C] dihydrotetra-benzazine. The site was pretreated with a biocompatible angiogenic scaffold, which was found to significantly improve engraftment versus control models. PET imaging visualized and quantified the islet mass and also correlated with the maintenance of normoglycemia by the islet graft [226].

In conclusion, imaging of islet cell mass has made significant strides in the past few years as novel areas of research develop all while new ones continue to spring up. And while such research gives enthusiasm for the potential to improve the evasive *in vivo* monitoring of islet survival and functionality, there is still some work required to establish a more reproducible and readily universally applicable modality to have a clinical impact.

10. Autotransplantation for Chronic Pancreatitis

The concept of islet autotransplantation following pancreatic resection as a surgical treatment option in patients with chronic pancreatitis first developed at the University of

Minnesota (UMN) in the 1970s, when Najarian et al. indicated therapeutic value in its role to relieve the pain in this patient population [17, 282, 283]. However, the side effect of diabetes must be taken into consideration when considering it as an alternative [17]. This has an important role in considering when to revert to surgery for treatment, as delaying in patients with chronic pancreatitis may lead to progressive damage of the pancreas and a subsequent decline in islet yield [284, 285].

In a recent retrospective study, it was found that up to 80% of patients had reduced or eliminated the need for narcotics [213]. Pain relief is obtained in most patients, and health-related quality of life is significantly improved [286–291]. A UMN analysis showed that nearly 95% of adult patients had less pain following surgery [292]. Insulin independence is preserved long term in about one-third of patients, with another third having sufficient beta-cell function so that the resulting diabetes is mild and easily controlled [287]. While there is a decline in graft function over time, long-term insulin secretion remains evident and may protect against long-term diabetic complications [293].

In a case study by Illouz et al., a patient suffering from chronic pancreatitis for more than two years and abnormal glucose tolerance test underwent pancreatectomy and islet autotransplantation and remains insulin independent 5 years after transplantation with less than 1,000 IEQ/kg body weight [294]. Interestingly, the same group has shown that no significant correlation exists between the number of islets transplanted and insulin independence. However, this may be as a result of differences in the etiology of chronic pancreatitis, such as secondary to chronic alcoholism [295]. This was in contrast to the University of Minnesota series [296]. Altogether, it is clear that islet graft function and efficacy following autotransplantation are greater, even with the presence of a lower β -cell mass [296, 297].

This same procedure has also been shown to have success in the pediatric population [298–300]. At the University of Minnesota, in 18 patients surveyed under the age of 18 suffering from chronic pancreatitis who underwent pancreatectomy and islet autotransplantation, only 7 were on narcotics, and 10 were insulin independent at 1 year. They concluded that the severity of diabetes may be reduced in three-fourths of patients, with higher graft efficacy in younger patients [298]. They also have identified that in the pediatric population, as in adults, performing the procedure early in the disease course is best to preserve islet cell mass and that preoperative measurement of fasting plasma glucose is useful for predicting islet yield [301, 302]. Furthermore, any surgical procedures prior to pancreatic resection should be avoided [299, 302].

11. Conclusion

Islet cell transplantation for the treatment of diabetes mellitus has made remarkable strides in its evolution towards truly becoming an alternative treatment to intensive medical therapy and pancreas transplantation. However, as highlighted in this paper, while barriers are identified

and advancements are made, progress remains until it may be considered a more efficacious and viable alternative to those already established. Having identified several areas that serve against islet survival in the peritransplant period, there is reason to remain optimistic that new therapies and protocols will be implemented and, thus, aid towards the gradual improvement of islet graft efficacy over the short and long course. In the end, we can hope that islet cell transplantation will serve to prevent the debilitating complications of diabetes mellitus and lead our patients to healthier lives.

Conflict of Interests

No competing financial interests exist.

References

- [1] C. Jahansouz, S. C. Kumer, M. Ellenbogen, and K. L. Brayman, "Evolution of β -cell replacement therapy in diabetes mellitus: pancreas transplantation," *Diabetes Technology and Therapeutics*, vol. 13, no. 3, pp. 395–418, 2011.
- [2] National Institute of Diabetes and Digestive and Kidney Diseases, *National Diabetes Statistics, 2007 Fact Sheet*, National Institutes of Health, Bethesda, Md, USA, 2008.
- [3] G. Bruno, G. Novelli, F. Panero et al., "The incidence of type 1 diabetes is increasing in both children and young adults in Northern Italy: 1984–2004 temporal trends," *Diabetologia*, vol. 52, no. 12, pp. 2531–2535, 2009.
- [4] C. C. Patterson, G. G. Dahlquist, E. Gyürüs, A. Green, and G. Soltész, "Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study," *The Lancet*, vol. 373, no. 9680, pp. 2027–2033, 2009.
- [5] P. Jarosz-Chobot, J. Polanska, A. Szadkowska et al., "Rapid increase in the incidence of type 1 diabetes in Polish children from 1989 to 2004, and predictions for 2010 to 2025," *Diabetologia*, vol. 54, no. 3, pp. 508–515, 2011.
- [6] T. Sella, A. Shoshan, I. Goren et al., "A retrospective study of the incidence of diagnosed Type 1 diabetes among children and adolescents in a large health organization in Israel, 2000–2008," *Diabetic Medicine*, vol. 28, no. 1, pp. 48–53, 2011.
- [7] G. Bruno and A. Landi, "Epidemiology and costs of diabetes," *Transplantation Proceedings*, vol. 43, no. 1, pp. 327–329, 2011.
- [8] G. Bruno, L. Karaghiosoff, F. Merletti et al., "The impact of diabetes on prescription drug costs: the population-based Turin study," *Diabetologia*, vol. 51, no. 5, pp. 795–801, 2008.
- [9] K. M. Gillespie, "Type 1 diabetes: pathogenesis and prevention," *Canadian Medical Association Journal*, vol. 175, no. 2, pp. 165–170, 2006.
- [10] M. Knip, "Environmental triggers and determinants of beta-cell autoimmunity and Type 1 diabetes," *Reviews in Endocrine and Metabolic Disorders*, vol. 4, no. 3, pp. 213–223, 2003.
- [11] M. Knip, R. Veijola, S. M. Virtanen, H. Hyöty, O. Vaarala, and H. K. Åkerblom, "Environmental triggers and determinants of type 1 diabetes," *Diabetes*, vol. 54, supplement 2, pp. S125–S136, 2005.
- [12] J. Von Mering and O. Minkowski, "Diabetes mellitus nach Pankreasextirpation," *Archiv für Experimentelle Pathologie und Pharmakologie*, vol. 26, pp. 371–387, 1890.
- [13] D. A. McClusky III, L. J. Skandalakis, G. L. Colborn, and J. E. Skandalakis, "Harbinger or hermit? Pancreatic anatomy and surgery through the ages—Part 3," *World Journal of Surgery*, vol. 26, no. 12, pp. 1512–1524, 2002.
- [14] W. D. Kelly, R. C. Lillehei, F. K. Merkel, Y. Idezuki, and F. G. Goetz, "Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy," *Surgery*, vol. 61, no. 6, pp. 827–837, 1967.
- [15] S. Misler, "The isolated pancreatic islet as a micro-organ and its transplantation to cure diabetes: celebrating the legacy of Paul Lacy," *Islets*, vol. 2, no. 4, pp. 210–224, 2010.
- [16] R. P. Robertson, "Islet transplantation a decade later and strategies for filling a half-full glass," *Diabetes*, vol. 59, no. 6, pp. 1285–1291, 2010.
- [17] J. S. Najarian, D. E. R. Sutherland, and D. Baumgartner, "Total or near total pancreatectomy and islet autotransplantation for treatment of chronic pancreatitis," *Annals of Surgery*, vol. 192, no. 4, pp. 526–542, 1980.
- [18] R. P. Robertson, C. Davis, J. Larsen, R. Stratta, and D. E. Sutherland, "Pancreas and islet transplantation in type 1 diabetes," *Diabetes Care*, vol. 29, no. 4, p. 935, 2006.
- [19] S. Moskalewski, "Beginning of pancreatic islet isolation by collagenase digestion," *General and Comparative Endocrinology*, vol. 44, pp. 342–353, 1965.
- [20] W. F. Ballinger and P. E. Lacy, "Transplantation of intact pancreatic islets in rats," *Surgery*, vol. 72, no. 2, pp. 175–186, 1972.
- [21] C. R. Reckard, M. M. Ziegler, and C. F. Barker, "Physiological and immunological consequences of transplanting isolated pancreatic islets," *Surgery*, vol. 74, no. 1, pp. 91–99, 1973.
- [22] C. B. Kemp, M. J. Knight, and D. W. Scharp, "Effect of transplantation site on the results of pancreatic islet isografts in diabetic rats," *Diabetologia*, vol. 9, no. 6, pp. 486–491, 1973.
- [23] D. W. Scharp, J. J. Murphy, and W. T. Newton, "Transplantation of islets of Langerhans in diabetic rhesus monkeys," *Surgery*, vol. 77, no. 1, pp. 100–105, 1975.
- [24] V. Mirkovitch and M. Campiche, "Successful intrasplenic autotransplantation of pancreatic tissue in totally pancreatectomized dogs," *Transplantation*, vol. 21, no. 3, pp. 265–269, 1976.
- [25] G. J. Kretschmer, D. E. Sutherland, A. J. Matas, L. Olson, and J. S. Najarian, "Islet autotransplantation in pancreatectomized dogs: liver vs spleen as a site of pancreatic tissue implantation," *Surgical Forum*, vol. 28, pp. 292–294, 1977.
- [26] D. G. Mehigan, G. D. Zuidema, J. C. Eggleston, and J. L. Cameron, "Pancreatic islet autotransplantation: results in dogs with chronic duct ligation," *American Journal of Surgery*, vol. 139, no. 2, pp. 170–174, 1980.
- [27] D. G. Mehigan, G. D. Zuidema, and J. L. Cameron, "Pancreatic islet transplantation in dogs. Critical factors in technique," *American Journal of Surgery*, vol. 141, no. 2, pp. 208–212, 1981.
- [28] D. E. R. Sutherland, P. F. Gores, A. C. Farney et al., "Evolution of kidney, pancreas, and islet transplantation for patients with diabetes at the University of Minnesota," *American Journal of Surgery*, vol. 166, no. 5, pp. 456–491, 1993.
- [29] F. Largiader, E. Kolb, and U. Binswanger, "A long-term functioning human pancreatic islet allotransplant," *Transplantation*, vol. 29, no. 1, pp. 76–77, 1980.
- [30] D. W. Scharp, P. E. Lacy, J. V. Santiago et al., "Insulin independence after islet transplantation into type I diabetic patient," *Diabetes*, vol. 39, no. 4, pp. 515–518, 1990.
- [31] C. Succi, L. Falqui, A. M. Davalli et al., "Fresh human islet transplantation to replace pancreatic endocrine function in type 1 diabetic patients—report of six cases," *Acta Diabetologica*, vol. 28, no. 2, pp. 151–157, 1991.

- [32] A. G. Tzakis, C. Ricordi, R. Alejandro et al., "Pancreatic islet transplantation after upper abdominal exenteration and liver replacement," *The Lancet*, vol. 336, no. 8712, pp. 402–405, 1990.
- [33] G. L. Warnock, N. M. Kneteman, E. Ryan, R. E. A. Seelis, A. Rabinovitch, and R. V. Rajotte, "Normoglycaemia after transplantation of freshly isolated and cryopreserved pancreatic islets in Type 1 (insulin-dependent) diabetes mellitus," *Diabetologia*, vol. 34, no. 1, pp. 55–58, 1991.
- [34] P. F. Gores, J. S. Najarian, E. Stephanian, J. J. Lloveras, S. L. Kelley, and D. E. R. Sutherland, "Insulin independence in type I diabetes after transplantation of unpurified islets from single donor with 15-deoxyspergualin," *The Lancet*, vol. 341, no. 8836, pp. 19–21, 1993.
- [35] B. J. Hering, M. O. Brendel, A. O. Schultz, B. Schultz, and R. G. Bretzel, *International islet Transplant Registry Newsletter*, vol. 6, pp. 1–20, 1996.
- [36] B. J. Hering, R. G. Bretzel, U. T. Hopt et al., "New protocol toward prevention of early human islet allograft failure," *Transplantation Proceedings*, vol. 26, no. 2, pp. 570–571, 1994.
- [37] B. J. Hering, W. Ernst, M. Eckhard, H. Branshorst, H. Jahr, and R. Grossman, "Improved survival of single donor islet allografts in 1DDM recipients by refined peritransplant management," *Diabetes*, vol. 46, supplement 1, p. 64, 1997.
- [38] A. Secchi, C. Socci, P. Maffi et al., "Islet transplantation in IDDM patients," *Diabetologia*, vol. 40, no. 2, pp. 225–231, 1997.
- [39] F. Bertuzzi, F. Grohovaz, P. Maffi et al., "Successful transplantation of human islets in recipients bearing a kidney graft," *Diabetologia*, vol. 45, no. 1, pp. 77–84, 2002.
- [40] T. Titus, L. Badet, and D. W. Gray, "Islet cell transplantation for insulin-dependant diabetes mellitus: perspectives from the present and prospects for the future," *Expert Reviews in Molecular Medicine*, vol. 2, no. 6, pp. 1–28, 2000.
- [41] N. S. Kenyon, A. Ranunco, M. Masetti, M. Chatzipetrou, and C. Ricordi, "Islet transplantation: present and future perspectives," *Diabetes/Metabolism Reviews*, vol. 14, no. 4, pp. 303–313, 1998.
- [42] B. Hering and C. Ricordi, "Islet transplantation for patients with type 1 diabetes," *Graft*, vol. 2, pp. 12–27, 1999.
- [43] A. M. J. Shapiro, J. R. T. Lakey, E. A. Ryan et al., "Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen," *New England Journal of Medicine*, vol. 343, no. 4, pp. 230–238, 2000.
- [44] A. M. J. Shapiro, C. Ricordi, B. J. Hering et al., "International trial of the Edmonton protocol for islet transplantation," *New England Journal of Medicine*, vol. 355, no. 13, pp. 1318–1330, 2006.
- [45] The CITR Coordinating Center and Investigators, "The collaborative islet transplant registry 2009 annual report," 2009, <http://www.citrregistry.org/reports/reports.htm>.
- [46] Y. Kuroda, T. Kawamura, Y. Suzuki, H. Fujiwara, K. Yamamoto, and Y. Saitoh, "A new, simple method for cold storage of the pancreas using perfluorochemical," *Transplantation*, vol. 46, no. 3, pp. 457–460, 1988.
- [47] T. Kin, "Islet isolation for clinical transplantation," *Advances in Experimental Medicine and Biology*, vol. 654, pp. 683–710, 2010.
- [48] A. Morita, Y. Kuroda, Y. Fujino, Y. Tanioka, Y. Ku, and Y. Saitoh, "Assessment of pancreas graft viability preserved by a two-layer (University of Wisconsin solution/perfluorochemical) method after significant warm ischemia," *Transplantation*, vol. 55, no. 3, pp. 667–669, 1993.
- [49] Y. Kim, Y. Kuroda, Y. Tanioka et al., "Recovery of pancreatic tissue perfusion and ATP tissue level after reperfusion in canine pancreas grafts preserved by the two-layer method," *Pancreas*, vol. 14, no. 3, pp. 285–289, 1997.
- [50] C. Ricordi, P. E. Lacy, E. H. Finke, B. J. Olack, and D. W. Scharp, "Automated method for isolation of human pancreatic islets," *Diabetes*, vol. 37, no. 4, pp. 413–420, 1988.
- [51] P. E. Lacy and M. Kostianovsky, "Method for the isolation of intact islets of Langerhans from the rat pancreas," *Diabetes*, vol. 16, no. 1, pp. 35–39, 1967.
- [52] S. Moskalewski, "Isolation and culture of the islets of Langerhans of the guinea pig," *General and Comparative Endocrinology*, vol. 5, no. 3, pp. 342–353, 1965.
- [53] E. Linetsky, R. Bottino, R. Lehmann, R. Alejandro, L. Inverardi, and C. Ricordi, "Improved human islet isolation using a new enzyme blend, liberase," *Diabetes*, vol. 46, no. 7, pp. 1120–1123, 1997.
- [54] B. J. Olack, C. J. Swanson, T. K. Howard, and T. Mohanakumar, "Improved method for the isolation and purification of human islets of langerhans using Liberase enzyme blend," *Human Immunology*, vol. 60, no. 12, pp. 1303–1309, 1999.
- [55] R. Alejandro, F. B. Barton, B. J. Hering, and S. Wease, "2008 Update from the collaborative islet transplant registry," *Transplantation*, vol. 86, no. 12, pp. 1783–1788, 2008.
- [56] T. Saito, T. Anazawa, M. Gotoh et al., "Actions of the Japanese pancreas and islet transplantation association regarding transplanted human islets isolated using liberase HI," *Transplantation Proceedings*, vol. 42, no. 10, pp. 4213–4216, 2010.
- [57] J. Caballero-Corbalán, H. Brandhorst, S. Asif et al., "Mammalian tissue-free liberase: a new GMP-graded enzyme blend for human islet isolation," *Transplantation*, vol. 90, no. 3, pp. 332–333, 2010.
- [58] M. Shimoda, H. Noguchi, B. Naziruddin et al., "Assessment of human islet isolation with four different collagenases," *Transplantation Proceedings*, vol. 42, no. 6, pp. 2049–2051, 2010.
- [59] D. M. Nikolic, P. B. Djordjevic, V. D. Sreckovic et al., "Comparative analysis of collagenase XI and liberase HI for the isolation of human pancreatic islets," *Hepato-Gastroenterology*, vol. 57, no. 104, pp. 1573–1578, 2010.
- [60] D. O'Gorman, T. Kin, S. Imes, R. Pawlick, P. Senior, and A. M. J. Shapiro, "Comparison of human islet isolation outcomes using a new mammalian tissue-free enzyme versus collagenase NB-1," *Transplantation*, vol. 90, no. 3, pp. 255–259, 2010.
- [61] H. Brandhorst, A. Friberg, B. Nilsson et al., "Large-scale comparison of liberase HI and collagenase NB1 utilized for human islet Isolation," *Cell Transplantation*, vol. 19, no. 1, pp. 3–8, 2010.
- [62] G. L. Szot, M. R. Lee, M. M. Tavakol et al., "Successful clinical islet isolation using a GMP-manufactured collagenase and neutral protease," *Transplantation*, vol. 88, no. 6, pp. 753–756, 2009.
- [63] J. Caballero-Corbalán, A. S. Friberg, H. Brandhorst et al., "Vitacyte collagenase HA: a novel enzyme blend for efficient human islet isolation," *Transplantation*, vol. 88, no. 12, pp. 1400–1402, 2009.
- [64] M. Nacher, V. Barceló, J. Escoriza, G. Joanny, M. Núñez-Ollé, and E. Montanya, "Optimization of human pancreatic islet isolation with a newly designed cooling system for COBE 2991," *Transplantation Proceedings*, vol. 41, no. 6, pp. 2202–2203, 2009.
- [65] S. P. Lake, P. D. Bassett, A. Larkins et al., "Large-scale purification of human islets utilizing discontinuous albumin

- gradient on IBM 2991 cell separator," *Diabetes*, vol. 38, supplement 1, pp. 143–145, 1989.
- [66] H. Noguchi, "Pancreatic islet transplantation," *World Journal of Gastrointestinal Surgery*, vol. 1, no. 1, pp. 16–20, 2009.
- [67] B. Olack, C. Swanson, M. McLearn, J. Longwith, D. Scharp, and P. E. Lacy, "Islet purification using Euro-Ficoll gradients," *Transplantation Proceedings*, vol. 23, no. 1, pp. 774–776, 1991.
- [68] H. Noguchi, T. Ikemoto, B. Naziruddin et al., "Iodixanol-controlled density gradient during islet purification improves recovery rate in human islet isolation," *Transplantation*, vol. 87, no. 11, pp. 1629–1635, 2009.
- [69] T. Min, L. Yi, Z. Chao et al., "Superiority of visipaque (iodixanol)-controlled density gradient over ficoll-400 in adult porcine islet purification," *Transplantation Proceedings*, vol. 42, no. 5, pp. 1825–1829, 2010.
- [70] A. Mita, C. Ricordi, A. Miki et al., "Purification method using iodixanol (OptiPrep)-based density gradient significantly reduces cytokine chemokine production from human islet preparations, leading to prolonged β -cell survival during pretransplantation culture," *Transplantation Proceedings*, vol. 41, no. 1, pp. 314–315, 2009.
- [71] A. Mita, C. Ricordi, S. Messinger et al., "Antiproinflammatory effects of iodixanol (OptiPrep)-based density gradient purification on human islet preparations," *Cell Transplantation*, vol. 19, no. 12, pp. 1537–1546, 2010.
- [72] J. Carter, S. Karmiol, M. Nagy et al., "Pretransplant islet culture: a comparison of four serum-free media using a murine model of islet transplantation," *Transplantation Proceedings*, vol. 37, no. 8, pp. 3446–3449, 2005.
- [73] H. Noguchi, B. Naziruddin, M. Shimoda et al., "Comparison of fresh and cultured islets from human and porcine pancreata," *Transplantation Proceedings*, vol. 42, no. 6, pp. 2084–2086, 2010.
- [74] M. A. Holmes, H. A. Clayton, D. R. Chadwick, P. R. F. Bell, N. J. M. London, and R. F. L. James, "Functional studies of rat, porcine, and human pancreatic islets cultured in ten commercially available media," *Transplantation*, vol. 60, no. 8, pp. 854–860, 1995.
- [75] H. Takahashi, M. Goto, N. Ogawa et al., "Superiority of fresh islets compared with cultured islets," *Transplantation Proceedings*, vol. 41, no. 1, pp. 350–351, 2009.
- [76] H. Takahashi, M. Goto, N. Ogawa et al., "Influence of a current style of culture on the quality of isolated pancreatic islets," *Transplantation Proceedings*, vol. 40, no. 2, pp. 358–359, 2008.
- [77] S. H. Ihm, I. Matsumoto, H. J. Zhang, J. D. Ansite, and B. J. Hering, "Effect of short-term culture on functional and stress-related parameters in isolated human islets," *Transplant International*, vol. 22, no. 2, pp. 207–216, 2009.
- [78] B. J. Hering, R. Kandaswamy, J. D. Ansite et al., "Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes," *Journal of the American Medical Association*, vol. 293, no. 7, pp. 830–835, 2005.
- [79] B. J. Hering, R. Kandaswamy, J. V. Harmon et al., "Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody," *American Journal of Transplantation*, vol. 4, no. 3, pp. 390–401, 2004.
- [80] H. Noguchi, B. Naziruddin, A. Jackson et al., "Low-temperature preservation of isolated islets is superior to conventional islet culture before islet transplantation," *Transplantation*, vol. 89, no. 1, pp. 47–54, 2010.
- [81] B. Kuttler, A. Hartmann, and H. Wanka, "Long-term culture of islets abrogates cytokine-induced or lymphocyte-induced increase of antigen expression on β cells," *Transplantation*, vol. 74, no. 4, pp. 440–445, 2002.
- [82] J. F. Markmann, J. Tomaszewski, A. M. Posselt et al., "The effect of islet cell culture in vitro at 24°C on graft survival and MHC antigen expression," *Transplantation*, vol. 49, no. 2, pp. 272–277, 1990.
- [83] S. Cabric, J. Sanchez, T. Lundgren et al., "Islet surface heparinization prevents the instant blood-mediated inflammatory reaction in islet transplantation," *Diabetes*, vol. 56, no. 8, pp. 2008–2015, 2007.
- [84] T. Kin, P. Senior, D. O'Gorman, B. Richer, A. Salam, and A. M. J. Shapiro, "Risk factors for islet loss during culture prior to transplantation," *Transplant International*, vol. 21, no. 11, pp. 1029–1035, 2008.
- [85] G. Loganathan, R. K. Dawra, S. Pugazhenthai et al., "Culture of impure human islet fractions in the presence of alpha-1 antitrypsin prevents insulin cleavage and improves islet recovery," *Transplantation Proceedings*, vol. 42, no. 6, pp. 2055–2057, 2010.
- [86] C. Toso, M. McCall, J. Emamaullee et al., "Liraglutide, a long-acting human glucagon-like peptide 1 analogue, improves human islet survival in culture," *Transplant International*, vol. 23, no. 3, pp. 259–265, 2010.
- [87] P. E. Lacy, J. M. Davie, and E. H. Finke, "Prolongation of islet allograft survival following in vitro culture (24°C) and a single injection of ALS," *Science*, vol. 204, no. 4390, pp. 312–313, 1979.
- [88] J. R. T. Lakey, G. L. Warnock, N. M. Kneteman, Z. Ao, and R. V. Rajotte, "Effects of pre-cryopreservation culture on human islet recovery and in vitro function," *Transplantation Proceedings*, vol. 26, no. 2, p. 820, 1994.
- [89] D. Brandhorst, H. Brandhorst, B. J. Hering, and R. G. Bretzel, "Long-term survival, morphology and in vitro function of isolated pig islets under different culture conditions," *Transplantation*, vol. 67, no. 12, pp. 1533–1541, 1999.
- [90] K. K. Papas, T. M. Suszynski, and C. K. Colton, "Islet assessment for transplantation," *Current Opinion in Organ Transplantation*, vol. 14, no. 6, pp. 674–682, 2009.
- [91] H. J. Kissler, J. C. Niland, B. Olack et al., "Validation of methodologies for quantifying isolated human islets: an islet cell resources study," *Clinical Transplantation*, vol. 24, no. 2, pp. 236–242, 2010.
- [92] N. Niclauss, A. Sgroi, P. Morel et al., "Computer-assisted digital image analysis to quantify the mass and purity of isolated human islets before transplantation," *Transplantation*, vol. 86, no. 11, pp. 1603–1609, 2008.
- [93] I. R. Sweet, G. Khalil, A. R. Wallen et al., "Continuous measurement of oxygen consumption by pancreatic islets," *Diabetes Technology and Therapeutics*, vol. 4, no. 5, pp. 661–672, 2002.
- [94] I. R. Sweet, M. Gilbert, R. Jensen et al., "Glucose stimulation of cytochrome C reduction and oxygen consumption as assessment of human islet quality," *Transplantation*, vol. 80, no. 8, pp. 1003–1011, 2005.
- [95] I. R. Sweet, M. Gilbert, S. Scott et al., "Glucose-stimulated increment in oxygen consumption rate as a standardized test of human islet quality," *American Journal of Transplantation*, vol. 8, no. 1, pp. 183–192, 2008.
- [96] K. K. Papas, C. K. Colton, R. A. Nelson et al., "Human islet oxygen consumption rate and DNA measurements predict diabetes reversal in nude mice," *American Journal of Transplantation*, vol. 7, no. 3, pp. 707–713, 2007.
- [97] K. K. Papas, C. K. Colton, A. Qipo et al., "Prediction of marginal mass required for successful islet transplantation," *Journal of Investigative Surgery*, vol. 23, no. 1, pp. 28–34, 2010.

- [98] M. Goto, J. Holgersson, M. Kumagai-Braesch, and O. Korsgren, "The ADP/ATP ratio: a novel predictive assay for quality assessment of isolated pancreatic islets," *American Journal of Transplantation*, vol. 6, no. 10, pp. 2483–2487, 2006.
- [99] D. A. Bradbury, T. D. Simmons, K. J. Slater, and S. P. M. Crouch, "Measurement of the ADP:ATP ratio in human leukaemic cell lines can be used as an indicator of cell viability, necrosis and apoptosis," *Journal of Immunological Methods*, vol. 240, no. 1–2, pp. 79–92, 2000.
- [100] M. V. Zamaraeva, R. Z. Sabirov, E. Maeno, Y. Ando-Akatsuka, S. V. Bessonova, and Y. Okada, "Cells die with increased cytosolic ATP during apoptosis: a bioluminescence study with intracellular luciferase," *Cell Death and Differentiation*, vol. 12, no. 11, pp. 1390–1397, 2005.
- [101] M. S. Hanson, E. E. Park, M. L. Sears et al., "A simplified approach to human islet quality assessment," *Transplantation*, vol. 89, no. 10, pp. 1178–1188, 2010.
- [102] The CITR Coordinating Center and Investigators, "The collaborative islet transplant registry 2008 annual report," 2008, <http://www.citregistry.org/reports/reports.htm>.
- [103] A. C. Gruessner, D. E. R. Sutherland, and R. W. G. Gruessner, "Pancreas transplantation in the United States: a review," *Current Opinion in Organ Transplantation*, vol. 15, no. 1, pp. 93–101, 2010.
- [104] Organ Procurement and Transplantation Network, April 2010, http://optn.transplant.hrsa.gov/data/request_main.asp.
- [105] K. P. McCullough, D. S. Keith, K. H. Meyer, P. G. Stock, K. L. Brayman, and A. B. Leichtman, "Kidney and pancreas transplantation in the United States, 1998–2007: access for patients with diabetes and end-stage renal disease," *American Journal of Transplantation*, vol. 9, no. 4, pp. 894–906, 2009.
- [106] J. Speight, M. D. Reaney, A. J. Woodcock, R. M. Smith, and J. A. M. Shaw, "Patient-reported outcomes following islet cell or pancreas transplantation (alone or after kidney) in Type 1 diabetes: a systematic review," *Diabetic Medicine*, vol. 27, no. 7, pp. 812–822, 2010.
- [107] C. Toso, R. Pawlick, S. Lacotte et al., "Detecting rejection after mouse islet transplantation utilizing islet protein-stimulated ELISPOT," *Cell Transplantation*, vol. 20, no. 6, pp. 955–962, 2011.
- [108] B. W. Paty, E. A. Ryan, A. M. J. Shapiro, J. R. T. Lakey, and R. P. Robertson, "Intrahepatic islet transplantation in type 1 diabetic patients does not restore hypoglycemic hormonal counterregulation or symptom recognition after insulin independence," *Diabetes*, vol. 51, no. 12, pp. 3428–3434, 2002.
- [109] M. R. Rickels, M. H. Schutta, R. Mueller et al., "Glycemic thresholds for activation of counterregulatory hormone and symptom responses in islet transplant recipients," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 3, pp. 873–879, 2007.
- [110] M. R. Rickels, M. H. Schutta, J. F. Markmann, C. F. Barker, A. Naji, and K. L. Teff, "β-Cell function following human islet transplantation for type 1 diabetes," *Diabetes*, vol. 54, no. 1, pp. 100–106, 2005.
- [111] R. G. Bretzel, H. Jahr, M. Eckhard, I. Martin, D. Winter, and M. D. Brendel, "Islet cell transplantation today," *Langenbeck's Archives of Surgery*, vol. 392, no. 3, pp. 239–253, 2007.
- [112] J. R. T. Lakey, T. Kin, G. L. Warnock et al., "Long-term graft function after allogeneic islet transplantation," *Cell Transplantation*, vol. 16, no. 4, pp. 441–446, 2007.
- [113] P. Cure, A. Pileggi, T. Froud et al., "Improved metabolic control and quality of life in seven patients with type 1 diabetes following islet after kidney transplantation," *Transplantation*, vol. 85, no. 6, pp. 801–812, 2008.
- [114] G. L. Warnock, R. M. Meloche, D. Thompson et al., "Improved human pancreatic islet isolation for a prospective cohort study of islet transplantation vs best medical therapy in type 1 diabetes mellitus," *Archives of Surgery*, vol. 140, no. 8, pp. 735–744, 2005.
- [115] R. Poggioli, G. Enfield, S. Messinger et al., "Nutritional status and behavior in subjects with type 1 diabetes, before and after islet transplantation," *Transplantation*, vol. 85, no. 4, pp. 501–506, 2008.
- [116] D. M. Thompson, I. S. Begg, C. Harris et al., "Reduced progression of diabetic retinopathy after islet cell transplantation compared with intensive medical therapy," *Transplantation*, vol. 85, no. 10, pp. 1400–1405, 2008.
- [117] T. C. Lee, N. R. Barshes, C. A. O'Mahony et al., "The effect of pancreatic islet transplantation on progression of diabetic retinopathy and neuropathy," *Transplantation Proceedings*, vol. 37, no. 5, pp. 2263–2265, 2005.
- [118] P. Fiorina, F. Folli, P. Maffi et al., "Islet transplantation improves vascular diabetic complications in patients with diabetes who underwent kidney transplantation: a comparison between kidney-pancreas and kidney-alone transplantation," *Transplantation*, vol. 75, no. 8, pp. 1296–1301, 2003.
- [119] P. Fiorina, C. Gremizzi, P. Maffi et al., "Islet transplantation is associated with an improvement of cardiovascular function in type 1 diabetic kidney transplant patients," *Diabetes Care*, vol. 28, no. 6, pp. 1358–1365, 2005.
- [120] P. Fiorina, F. Folli, G. Zerbini et al., "Islet transplantation is associated with improvement of renal function among uremic patients with type I diabetes mellitus and kidney transplants," *Journal of the American Society of Nephrology*, vol. 14, no. 8, pp. 2150–2158, 2003.
- [121] U. Del Carro, P. Fiorina, S. Amadio et al., "Evaluation of polyneuropathy markers in type 1 diabetic kidney transplant patients and effects of islet transplantation: neurophysiological and skin biopsy longitudinal analysis," *Diabetes Care*, vol. 30, no. 12, pp. 3063–3069, 2007.
- [122] M. Naesens, D. R. J. Kuypers, and M. Sarwal, "Calcineurin inhibitor nephrotoxicity," *Clinical Journal of the American Society of Nephrology*, vol. 4, no. 2, pp. 481–508, 2009.
- [123] F. T. Nielsen, P. Ottosen, H. Starklint, and H. Dieperink, "Kidney function and morphology after short-term combination therapy with cyclosporine A, tacrolimus and sirolimus in the rat," *Nephrology Dialysis Transplantation*, vol. 18, no. 3, pp. 491–496, 2003.
- [124] Q. Su, L. Weber, M. Le Hir, G. Zenke, and B. Ryffel, "Nephrotoxicity of cyclosporin A and FK506: inhibition of calcineurin phosphatase," *Renal Physiology and Biochemistry*, vol. 18, no. 3, pp. 128–139, 1995.
- [125] J. C. Chen and P. Ma, "Mechanism of FK506-induced renal hypoperfusion and its reversion in rats," *Acta Pharmacologica Sinica*, vol. 22, no. 11, pp. 1034–1038, 2001.
- [126] R. J. T. Owen, E. A. Ryan, K. O'Kelly et al., "Percutaneous transhepatic pancreatic islet cell transplantation in type 1 diabetes mellitus: radiologic aspects," *Radiology*, vol. 229, no. 1, pp. 165–170, 2003.
- [127] P. Villiger, E. A. Ryan, R. Owen et al., "Prevention of bleeding after islet transplantation: lessons learned from a multivariate analysis of 132 cases at a single institution," *American Journal of Transplantation*, vol. 5, no. 12, pp. 2992–2998, 2005.
- [128] G. Maleux, P. Gillard, B. Keymeulen et al., "Feasibility, safety, and efficacy of percutaneous transhepatic injection of beta-cell grafts," *Journal of Vascular and Interventional Radiology*, vol. 16, no. 12, pp. 1693–1697, 2005.

- [129] P. Bucher, Z. Mathe, D. Bosco et al., "Morbidity associated with intraportal islet transplantation," *Transplantation Proceedings*, vol. 36, no. 4, pp. 1119–1120, 2004.
- [130] P. M. Campbell, P. A. Senior, A. Salam et al., "High risk of sensitization after failed islet transplantation," *American Journal of Transplantation*, vol. 7, no. 10, pp. 2311–2317, 2007.
- [131] N. Waugh, "Could fewer islet cells be transplant in type 1 diabetes?" *British Medical Journal*, vol. 321, no. 7275, p. 1534, 2000.
- [132] L. Badet, P. Y. Benhamou, A. Wojtusciszyn et al., "Expectations and strategies regarding islet transplantation: metabolic data from the GRAGIL 2 trial," *Transplantation*, vol. 84, no. 1, pp. 89–96, 2007.
- [133] P. Y. Benhamou, J. Oberholzer, C. Toso et al., "Human islet transplantation network for the treatment of Type I diabetes: first data from the Swiss-French GRAGIL consortium (1999–2000)," *Diabetologia*, vol. 44, no. 7, pp. 859–864, 2001.
- [134] L. Kessler, P. Bucher, L. Milliat-Guittard et al., "Influence of islet transportation on pancreatic islet allotransplantation in type 1 diabetic patients within the Swiss-French GRAGIL network," *Transplantation*, vol. 77, no. 8, pp. 1301–1304, 2004.
- [135] K. I. Rother and D. M. Harlan, "Challenges facing islet transplantation for the treatment of type 1 diabetes mellitus," *Journal of Clinical Investigation*, vol. 114, no. 7, pp. 877–883, 2004.
- [136] V. A. L. Huurman, R. Hilbrands, G. G. M. Pinkse et al., "Cellular islet autoimmunity associates with clinical outcome of islet cell transplantation," *PLoS ONE*, vol. 3, no. 6, Article ID e2435, 2008.
- [137] P. Monti, M. Scirpoli, P. Maffi et al., "Islet transplantation in patients with autoimmune diabetes induces homeostatic cytokines that expand autoreactive memory T cells," *Journal of Clinical Investigation*, vol. 118, no. 5, pp. 1806–1814, 2008.
- [138] S. Subramanian and D. L. Trencle, "Immunosuppressive agents: effects on glucose and lipid metabolism," *Endocrinology and Metabolism Clinics of North America*, vol. 36, no. 4, pp. 891–905, 2007.
- [139] T. Nir, D. A. Melton, and Y. Dor, "Recovery from diabetes in mice by β cell regeneration," *Journal of Clinical Investigation*, vol. 117, no. 9, pp. 2553–2561, 2007.
- [140] J. B. Redmon, L. K. Olson, M. B. Armstrong, M. J. Greene, and R. P. Robertson, "Effects of tacrolimus (FK506) on human insulin gene expression, insulin mRNA levels, and insulin secretion in HIT-T15 cells," *Journal of Clinical Investigation*, vol. 98, no. 12, pp. 2786–2793, 1996.
- [141] E. Oetjen, D. Baun, S. Beimesche et al., "Inhibition of human insulin gene transcription by the immunosuppressive drugs cyclosporin A and tacrolimus in primary, mature islets of transgenic mice," *Molecular Pharmacology*, vol. 63, no. 6, pp. 1289–1295, 2003.
- [142] B. W. Paty, J. S. Harmon, C. L. Marsh, and R. P. Robertson, "Inhibitory effects of immunosuppressive drugs on insulin secretion from HIT-T15 cells and Wistar rat islets," *Transplantation*, vol. 73, no. 3, pp. 353–357, 2002.
- [143] C. Ricordi, Y. Zeng, R. Alejandro et al., "In vivo effect of FK506 on human pancreatic islets," *Transplantation*, vol. 52, no. 3, pp. 519–522, 1991.
- [144] N. Rostambeigi, I. R. Lanza, P. P. Dzeja et al., "Unique cellular and mitochondrial defects mediate FK506-induced islet β -cell dysfunction," *Transplantation*, vol. 91, no. 6, pp. 615–623, 2011.
- [145] N. Niclauss, D. Bosco, P. Morel, L. Giovannoni, T. Berney, and G. Parnaud, "Rapamycin impairs proliferation of transplanted islet β cells," *Transplantation*, vol. 91, no. 7, pp. 714–722, 2011.
- [146] E. Zahr, R. D. Molano, A. Pileggi et al., "Rapamycin impairs β -cell proliferation in vivo," *Transplantation Proceedings*, vol. 40, no. 2, pp. 436–437, 2008.
- [147] E. Zahr, R. D. Molano, A. Pileggi et al., "Rapamycin impairs in vivo proliferation of islet beta-cells," *Transplantation*, vol. 84, no. 12, pp. 1576–1583, 2007.
- [148] C. T. Bussiere, J. R. T. Lakey, A. M. J. Shapiro, and G. S. Korbutt, "The impact of the mTOR inhibitor sirolimus on the proliferation and function of pancreatic islets and ductal cells," *Diabetologia*, vol. 49, no. 10, pp. 2341–2349, 2006.
- [149] V. Cantaluppi, L. Biancone, G. M. Romanazzi et al., "Antiangiogenic and immunomodulatory effects of rapamycin on islet endothelium: relevance for islet transplantation," *American Journal of Transplantation*, vol. 6, no. 11, pp. 2601–2611, 2006.
- [150] R. Gao, J. Ustinov, O. Korsgren, and T. Otonkoski, "Effects of immunosuppressive drugs on in vitro neogenesis of human islets: mycophenolate mofetil inhibits the proliferation of ductal cells," *American Journal of Transplantation*, vol. 7, no. 4, pp. 1021–1026, 2007.
- [151] J. D. Johnson, Z. Ao, P. Ao et al., "Different effects of FK506, rapamycin, and mycophenolate mofetil on glucose-stimulated insulin release and apoptosis in human islets," *Cell Transplantation*, vol. 18, no. 8, pp. 833–845, 2009.
- [152] K. A. Ghofaili, M. Fung, Z. Ao et al., "Effect of exenatide on β cell function after islet transplantation in type 1 diabetes," *Transplantation*, vol. 83, no. 1, pp. 24–28, 2007.
- [153] M. Fung, D. Thompson, R. J. Shapiro et al., "Effect of glucagon-like peptide-1 (7-37) on beta-cell function after islet transplantation in type 1 diabetes," *Diabetes Research and Clinical Practice*, vol. 74, no. 2, pp. 189–193, 2006.
- [154] T. Froud, R. N. Faradji, A. Pileggi et al., "The use of exenatide in islet transplant recipients with chronic allograft dysfunction: safety, efficacy, and metabolic effects," *Transplantation*, vol. 86, no. 1, pp. 36–45, 2008.
- [155] R. N. Faradji, T. Froud, S. Messinger et al., "Long-term metabolic and hormonal effects of exenatide on islet transplant recipients with allograft dysfunction," *Cell Transplantation*, vol. 18, no. 10–11, pp. 1247–1259, 2009.
- [156] R. N. Faradji, T. Tharavani, S. Messinger et al., "Long-term insulin independence and improvement in insulin secretion after supplemental islet infusion under exenatide and etanercept," *Transplantation*, vol. 86, no. 12, pp. 1658–1665, 2008.
- [157] A. Gangemi, P. Salehi, B. Hatipoglu et al., "Islet transplantation for brittle type 1 diabetes: the UIC protocol," *American Journal of Transplantation*, vol. 8, no. 6, pp. 1250–1261, 2008.
- [158] D. Mineo, J. Sageshima, G. W. Burke, and C. Ricordi, "Minimization and withdrawal of steroids in pancreas and islet transplantation," *Transplant International*, vol. 22, no. 1, pp. 20–37, 2009.
- [159] M. D. Bellin, R. Kandaswamy, J. Parkey et al., "Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes," *American Journal of Transplantation*, vol. 8, no. 11, pp. 2463–2470, 2008.
- [160] N. A. Turgeon, J. G. Avila, J. A. Cano et al., "Experience with a novel efalizumab-based immunosuppressive regimen to facilitate single donor islet cell transplantation," *American Journal of Transplantation*, vol. 10, no. 9, pp. 2082–2091, 2010.

- [161] S. Matsumoto, M. Takita, D. Chaussabel et al., "Improving efficacy of clinical islet transplantation with iodixanol based islet purification, thymoglobulin induction and blockage of IL-1-beta and TNF-alpha," *Cell Transplant*, vol. 20, no. 7, 2011.
- [162] A. M. Posselt, G. L. Szot, L. A. Frassetto et al., "Islet transplantation in type 1 diabetic patients using calcineurin inhibitor-free immunosuppressive protocols based on T-cell adhesion or costimulation blockade," *Transplantation*, vol. 90, no. 12, pp. 1595–1601, 2010.
- [163] S. Paraskevas, D. Maysinger, R. Wang, W. P. Duguid, and L. Rosenberg, "Cell loss in isolated human islets occurs by apoptosis," *Pancreas*, vol. 20, no. 3, pp. 270–276, 2000.
- [164] B. Gimi, L. Leoni, J. Oberholzer et al., "Functional MR micro-imaging of pancreatic β -cell activation," *Cell Transplantation*, vol. 15, no. 2, pp. 195–203, 2006.
- [165] T. Linn, J. Schmitz, I. Hauck-Schmalenberger et al., "Ischemia is linked to inflammation and induction of angiogenesis in pancreatic islets," *Clinical and Experimental Immunology*, vol. 144, no. 2, pp. 179–187, 2006.
- [166] P.-O. Carlsson, F. Palm, and G. Mattsson, "Low revascularization of experimentally transplanted human pancreatic islets," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 12, pp. 5418–5423, 2002.
- [167] M. Giuliani, W. Moritz, E. Bodmer et al., "Central necrosis in isolated hypoxic human pancreatic islets: evidence for post-isolation ischemia," *Cell Transplantation*, vol. 14, no. 1, pp. 67–76, 2005.
- [168] G. Miao, R. P. Ostrowski, J. Mace et al., "Dynamic production of hypoxia-inducible factor-1 α in early transplanted islets," *American Journal of Transplantation*, vol. 6, no. 11, pp. 2636–2643, 2006.
- [169] J. Cantley, S. T. Grey, P. H. Maxwell, and D. J. Withers, "The hypoxia response pathway and β -cell function," *Diabetes, Obesity and Metabolism*, vol. 12, supplement 2, pp. 159–167, 2010.
- [170] S. H. Ko, G. R. Ryu, S. Kim et al., "Inducible nitric oxide synthase-nitric oxide plays an important role in acute and severe hypoxic injury to pancreatic beta cells," *Transplantation*, vol. 85, no. 3, pp. 323–330, 2008.
- [171] J. Lau, J. Henriksnäs, J. Svensson, and P. O. Carlsson, "Oxygenation of islets and its role in transplantation," *Current Opinion in Organ Transplantation*, vol. 14, no. 6, pp. 688–693, 2009.
- [172] A. Riboulet-Chavey, F. Diraison, L. K. Siew, F. S. Wong, and G. A. Rutter, "Inhibition of AMP-activated protein kinase protects pancreatic β -cells from cytokine-mediated apoptosis and CD8⁺ T-cell-induced cytotoxicity," *Diabetes*, vol. 57, no. 2, pp. 415–423, 2008.
- [173] G. R. Ryu, M.-K. Lee, E. Lee et al., "Activation of AMP-activated protein kinase mediates acute and severe hypoxic injury to pancreatic beta cells," *Biochemical and Biophysical Research Communications*, vol. 386, no. 2, pp. 356–362, 2009.
- [174] S. K. Richards, L. E. Parton, I. Leclerc, G. A. Rutter, and R. M. Smith, "Over-expression of AMP-activated protein kinase impairs pancreatic β -cell function in vivo," *Journal of Endocrinology*, vol. 187, no. 2, pp. 225–235, 2005.
- [175] G. C. Weir and S. Bonner-Weir, "Five of stages of evolving β -cell dysfunction during progression to diabetes," *Diabetes*, vol. 53, supplement 3, pp. S16–S21, 2004.
- [176] X. Huang, D. J. Moore, R. J. Ketchum et al., "Resolving the conundrum of islet transplantation by linking metabolic dysregulation, inflammation, and immune regulation," *Endocrine Reviews*, vol. 29, no. 5, pp. 603–630, 2008.
- [177] S. A. Nanji and A. M. J. Shapiro, "Islet transplantation in patients with diabetes mellitus: choice of immunosuppression," *BioDrugs*, vol. 18, no. 5, pp. 315–328, 2004.
- [178] S. Merani, W. W. Truong, W. Hancock, C. C. Anderson, and A. M. J. Shapiro, "Chemokines and their receptors in islet allograft rejection and as targets for tolerance induction," *Cell Transplantation*, vol. 15, no. 4, pp. 295–309, 2006.
- [179] S. B. Dula, M. Jecmenica, R. Wu et al., "Evidence that low-grade systemic inflammation can induce islet dysfunction as measured by impaired calcium handling," *Cell Calcium*, vol. 48, no. 2-3, pp. 133–142, 2010.
- [180] C. Pflieger, N. C. Schloot, M. D. Brendel et al., "Circulating cytokines are associated with human islet graft function in type 1 diabetes," *Clinical Immunology*, vol. 138, no. 2, pp. 154–161, 2011.
- [181] M. Y. Donath, J. Störling, L. A. Berchtold, N. Billestrup, and T. Mandrup-Poulsen, "Cytokines and β -cell biology: from concept to clinical translation," *Endocrine Reviews*, vol. 29, no. 3, pp. 334–350, 2008.
- [182] Clinical islet Transplantation Consortium, "Clinical islet Transplantation Protocol (CIT '07)," 2010, http://www.ctsdmc.org/projects/cit/documents/CIT-07Protocol.Version.5.0_11Jan10.pdf
- [183] Q. Gao, L. L. Ma, X. Gao, W. Yan, P. Williams, and D. P. Yin, "TLR4 mediates early graft failure after intraportal islet transplantation," *American Journal of Transplantation*, vol. 10, no. 7, pp. 1588–1596, 2010.
- [184] B. Krüger, N. Yin, N. Zhang et al., "Islet-expressed TLR2 and TLR4 sense injury and mediate early graft failure after transplantation," *European Journal of Immunology*, vol. 40, no. 10, pp. 2914–2924, 2010.
- [185] N. Zhang, B. Krüger, G. Lal et al., "Inhibition of TLR4 signaling prolongs Treg-dependent murine islet allograft survival," *Immunology Letters*, vol. 127, no. 2, pp. 119–125, 2010.
- [186] A. Goldberg, M. Parolini, B. Y. Chin et al., "Toll-like receptor 4 suppression leads to islet allograft survival," *FASEB Journal*, vol. 21, no. 11, pp. 2840–2848, 2007.
- [187] Y. Lai, H. Brandhorst, H. Hossain et al., "Activation of NF κ B dependent apoptotic pathway in pancreatic islet cells by hypoxia," *Islets*, vol. 1, no. 1, pp. 19–25, 2009.
- [188] X. Hu, O. Nestic-Taylor, J. Qiu et al., "Activation of nuclear factor- κ B signaling pathway by interleukin-1 after hypoxia/ischemia in neonatal rat hippocampus and cortex," *Journal of Neurochemistry*, vol. 93, no. 1, pp. 26–37, 2005.
- [189] S. North, M. Moenner, and A. Bikfalvi, "Recent developments in the regulation of the angiogenic switch by cellular stress factors in tumors," *Cancer Letters*, vol. 218, no. 1, pp. 1–14, 2005.
- [190] F. Ortis, P. Pirot, N. Naamane et al., "Induction of nuclear factor- κ B and its downstream genes by TNF- α and IL-1 β has a pro-apoptotic role in pancreatic beta cells," *Diabetologia*, vol. 51, no. 7, pp. 1213–1225, 2008.
- [191] D. Melloul, "Role of NF-kappaB in beta-cell death," *Biochemical Society Transactions*, vol. 36, no. 3, pp. 334–339, 2008.
- [192] C. Chen, R. Moreno, B. Samikannu, R. G. Bretzel, M. L. Schmitz, and T. Linn, "Improved intraportal islet transplantation outcome by systemic IKK-beta inhibition: NF- κ B activity in pancreatic islets depends on oxygen availability," *American Journal of Transplantation*, vol. 11, no. 2, pp. 215–224, 2011.
- [193] N. Matsuoka, T. Itoh, H. Watarai et al., "High-mobility group box 1 is involved in the initial events of early loss of transplanted islets in mice," *Journal of Clinical Investigation*, vol. 120, no. 3, pp. 735–743, 2010.

- [194] L. Piemonti, B. E. Leone, R. Nano et al., "Human pancreatic islets produce and secrete MCP-1/CCL2: relevance in human islet transplantation," *Diabetes*, vol. 51, no. 1, pp. 55–65, 2002.
- [195] F. Bertuzzi, S. Marzorati, P. Maffi et al., "Tissue factor and CCL2/monocyte chemoattractant protein-1 released by human islets affect islet engraftment in type 1 diabetic recipients," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 11, pp. 5724–5728, 2004.
- [196] A. K. Cardozo, P. Proost, C. Gysemans, M. C. Chen, C. Mathieu, and D. L. Eizirik, "IL-1 β and IFN- γ induce the expression of diverse chemokines and IL-15 in human and rat pancreatic islet cells, and in islets from pre-diabetic NOD mice," *Diabetologia*, vol. 46, no. 2, pp. 255–266, 2003.
- [197] C. Ehrnfelt, M. Kumagai-Braesch, M. Uzunel, and J. Holgersson, "Adult porcine islets produce MCP-1 and recruit human monocytes in vitro," *Xenotransplantation*, vol. 11, no. 2, pp. 184–194, 2004.
- [198] A. P. Martin, S. Rankin, S. Pitchford, I. F. Charo, G. C. Furtado, and S. A. Lira, "Increased expression of CCL2 in insulin-producing cells of transgenic mice promotes mobilization of myeloid cells from the bone marrow, marked insulinitis, and diabetes," *Diabetes*, vol. 57, no. 11, pp. 3025–3033, 2008.
- [199] A. C. Ogliari, R. Caldara, C. Socci et al., "High levels of donor CCL2/MCP-1 predict graft-related complications and poor graft survival after kidney-pancreas transplantation," *American Journal of Transplantation*, vol. 8, no. 6, pp. 1303–1311, 2008.
- [200] Y. Saito, M. Goto, K. Maya et al., "Brain death in combination with warm ischemic stress during isolation procedures induces the expression of crucial inflammatory mediators in the isolated islets," *Cell Transplantation*, vol. 19, no. 6-7, pp. 775–782, 2010.
- [201] R. Melzi, A. Mercalli, V. Sordi et al., "Role of CCL2/MCP-1 in islet transplantation," *Cell Transplantation*, vol. 19, no. 8, pp. 1031–1046, 2010.
- [202] I. Lee, L. Wang, A. D. Wells et al., "Blocking the monocyte chemoattractant protein-1/CCR2 chemokine pathway induces permanent survival of islet allografts through a programmed death-1 ligand-1-dependent mechanism," *Journal of Immunology*, vol. 171, no. 12, pp. 6929–6935, 2003.
- [203] G. Chipitsyna, Q. Gong, C. F. Gray, Y. Haroon, E. Kamer, and H. A. Arafat, "Induction of monocyte chemoattractant protein-1 expression by angiotensin II in the pancreatic islets and β -cells," *Endocrinology*, vol. 148, no. 5, pp. 2198–2208, 2007.
- [204] B. Kutlu, M. I. Darville, A. K. Cardozo, and D. L. Eizirik, "Molecular regulation of monocyte chemoattractant protein-1 expression in pancreatic β -cells," *Diabetes*, vol. 52, no. 2, pp. 348–355, 2003.
- [205] P. Chhabra, K. Wang, Q. Zeng et al., "Adenosine A_{2A} agonist administration improves islet transplant outcome: evidence for the role of innate immunity in islet graft rejection," *Cell Transplantation*, vol. 19, no. 5, pp. 597–612, 2010.
- [206] T. Nitta, T. Itoh, N. Matsuoka et al., "Prevention of early loss of transplanted islets in the liver of mice by adenosine," *Transplantation*, vol. 88, no. 1, pp. 49–56, 2009.
- [207] A. Koh, P. Senior, A. Salam et al., "Insulin-heparin infusions peritransplant substantially improve single-donor clinical islet transplant success," *Transplantation*, vol. 89, no. 4, pp. 465–471, 2010.
- [208] V. Gupta, D. C. Wahoff, D. P. Rooney et al., "The defective glucagon response from transplanted intrahepatic pancreatic islets during hypoglycemia is transplantation site-determined," *Diabetes*, vol. 46, no. 1, pp. 28–33, 1997.
- [209] D. M. Kendall, D. P. Rooney, Y. F. C. Smets, L. S. Bolding, and R. P. Robertson, "Pancreas transplantation restores epinephrine response and symptom recognition during hypoglycemia in patients with long-standing type 1 diabetes and autonomic neuropathy," *Diabetes*, vol. 46, no. 2, pp. 249–257, 1997.
- [210] Z. Barrou, E. R. Seaquist, and R. P. Robertson, "Pancreas transplantation in diabetic humans normalizes hepatic glucose production during hypoglycemia," *Diabetes*, vol. 43, no. 5, pp. 661–666, 1994.
- [211] P. Diem, J. B. Redmon, M. Abid et al., "Glucagon, catecholamine and pancreatic polypeptide secretion in type 1 diabetic recipients of pancreas allografts," *Journal of Clinical Investigation*, vol. 86, no. 6, pp. 2008–2013, 1990.
- [212] H. Zhou, T. Zhang, M. Bogdani et al., "Intrahepatic glucose flux as a mechanism for defective intrahepatic islet α -cell response to hypoglycemia," *Diabetes*, vol. 57, no. 6, pp. 1567–1574, 2008.
- [213] R. P. Robertson, "Update on transplanting beta cells for reversing type 1 diabetes," *Endocrinology and Metabolism Clinics of North America*, vol. 39, no. 3, pp. 655–667, 2010.
- [214] P. O. Carlsson, F. Palm, A. Andersson, and P. Liss, "Chronically decreased oxygen tension in rat pancreatic islets transplanted under the kidney capsule," *Transplantation*, vol. 69, no. 5, pp. 761–766, 2000.
- [215] P. O. Carlsson, F. Palm, A. Andersson, and P. Liss, "Markedly decreased oxygen tension in transplanted rat pancreatic islets irrespective of the implantation site," *Diabetes*, vol. 50, no. 3, pp. 489–495, 2001.
- [216] D. Yin, J. W. Ding, J. Shen, L. Ma, M. Hara, and A. S. Chong, "Liver ischemia contributes to early islet failure following intraportal transplantation: benefits of liver ischemic-preconditioning," *American Journal of Transplantation*, vol. 6, no. 1, pp. 60–68, 2006.
- [217] S. Merani, C. Toso, J. Emamaullee, and A. M. J. Shapiro, "Optimal implantation site for pancreatic islet transplantation," *British Journal of Surgery*, vol. 95, no. 12, pp. 1449–1461, 2008.
- [218] H. I. Kim, J. E. Yu, C. G. Park, and S. J. Kim, "Comparison of four pancreatic islet implantation sites," *Journal of Korean Medical Science*, vol. 25, no. 2, pp. 203–210, 2010.
- [219] D. W. R. Gray, R. Sutton, P. McShane, M. Peters, and P. J. Morris, "Exocrine contamination impairs implantation of pancreatic islets transplanted beneath the kidney capsule," *Journal of Surgical Research*, vol. 45, no. 5, pp. 432–442, 1988.
- [220] K. C. Yang, C. C. Wu, Z. Qi, J. C. Chen, S. Sumi, and F. H. Lin, "Comparison of bioartificial pancreas performance in the bone marrow cavity and intramuscular space," *Archives of Medical Research*, vol. 41, no. 3, pp. 151–153, 2010.
- [221] K. C. Yang, C. C. Wu, Z. F. Kuo, C. Y. Yang, and F. H. Lin, "Intramedullary cavity as implantation site for bioartificial pancreas: preliminary in vivo study," *Transplantation Proceedings*, vol. 42, no. 7, pp. 2666–2668, 2010.
- [222] T. Lund, O. Korsgren, I. A. Aursnes, H. Scholz, and A. Foss, "Sustained reversal of diabetes following islet transplantation to striated musculature in the rat," *Journal of Surgical Research*, vol. 160, no. 1, pp. 145–154, 2010.
- [223] J. Svensson, J. Lau, M. Sandberg, and P.-O. Carlsson, "High vascular density and oxygenation of pancreatic islets transplanted in clusters into striated muscle," *Cell Transplantation*, vol. 20, no. 5, pp. 783–788, 2011.

- [224] G. Christoffersson, P.-O. Carlsson, and M. Phillipson, "Intramuscular islet transplantation promotes restored islet vascularity," *Islets*, vol. 3, no. 2, pp. 69–71, 2011.
- [225] G. Christoffersson, J. Henriksen, L. Johansson et al., "Clinical and experimental pancreatic islet transplantation to striated muscle: establishment of a vascular system similar to that in native islets," *Diabetes*, vol. 59, no. 10, pp. 2569–2578, 2010.
- [226] P. Witkowski, H. Sondermeijer, M. A. Hardy et al., "Islet grafting and imaging in a bioengineered intramuscular space," *Transplantation*, vol. 88, no. 9, pp. 1065–1074, 2009.
- [227] D. M. Salvay, C. B. Rives, X. Zhang et al., "Extracellular matrix protein-coated scaffolds promote the reversal of diabetes after extrahepatic islet transplantation," *Transplantation*, vol. 85, no. 10, pp. 1456–1464, 2008.
- [228] S. Sigrist, A. Mechine-Neuville, K. Mandes et al., "Influence of VEGF on the viability of encapsulated pancreatic rat islets after transplantation in diabetic mice," *Cell Transplantation*, vol. 12, no. 6, pp. 627–635, 2003.
- [229] S. Sigrist, A. Mechine-Neuville, K. Mandes et al., "Induction of angiogenesis in omentum with vascular endothelial growth factor: influence on the viability of encapsulated rat pancreatic islets during transplantation," *Journal of Vascular Research*, vol. 40, no. 4, pp. 359–367, 2003.
- [230] Y. Cheng, Y. F. Liu, J. L. Zhang, T. M. Li, and N. Zhao, "Elevation of vascular endothelial growth factor production and its effect on revascularization and function of graft islets in diabetic rats," *World Journal of Gastroenterology*, vol. 13, no. 20, pp. 2862–2866, 2007.
- [231] N. Zhang, A. Richter, J. Suriawinata et al., "Elevated vascular endothelial growth factor production in islets improves islet graft vascularization," *Diabetes*, vol. 53, no. 4, pp. 963–970, 2004.
- [232] Y. Lai, D. Schneider, A. Kizdzun et al., "Vascular endothelial growth factor increases functional β -cell mass by improvement of angiogenesis of isolated human and murine pancreatic islets," *Transplantation*, vol. 79, no. 11, pp. 1530–1536, 2005.
- [233] P. Stiegler, V. Matzi, E. Pierer et al., "Creation of a prevascularized site for cell transplantation in rats," *Xenotransplantation*, vol. 17, no. 5, pp. 379–390, 2010.
- [234] Y. Fumimoto, A. Matsuyama, H. Komoda et al., "Creation of a rich subcutaneous vascular network with implanted adipose tissue-derived stromal cells and adipose tissue enhances subcutaneous grafting of islets in diabetic mice," *Tissue Engineering C*, vol. 15, no. 3, pp. 437–444, 2009.
- [235] Y. Ohmura, M. Tanemura, N. Kawaguchi et al., "Combined transplantation of pancreatic islets and adipose tissue-derived stem cells enhances the survival and insulin function of islet grafts in diabetic mice," *Transplantation*, vol. 90, no. 12, pp. 1366–1373, 2010.
- [236] T. Ito, S. Itakura, I. Todorov et al., "Mesenchymal stem cell and islet co-transplantation promotes graft revascularization and function," *Transplantation*, vol. 89, no. 12, pp. 1438–1445, 2010.
- [237] I. R. Duprez, U. Johansson, B. Nilsson, O. Korsgren, and P. U. Magnusson, "Preparatory studies of composite mesenchymal stem cell islets for application in intraportal islet transplantation," *Uppsala Journal of Medical Sciences*, vol. 116, no. 1, pp. 8–17, 2011.
- [238] B. Longoni, E. Szilagyi, P. Quaranta et al., "Mesenchymal stem cells prevent acute rejection and prolong graft function in pancreatic islet transplantation," *Diabetes Technology & Therapeutics*, vol. 12, no. 6, pp. 435–446, 2010.
- [239] M. Figliuzzi, R. Cornolti, N. Perico et al., "Bone marrow-derived mesenchymal stem cells improve islet graft function in diabetic rats," *Transplantation Proceedings*, vol. 41, no. 5, pp. 1797–1800, 2009.
- [240] M. Shimoda, S. Chen, H. Noguchi, S. Matsumoto, and P. A. Grayburn, "In vivo non-viral gene delivery of human vascular endothelial growth factor improves revascularisation and restoration of euglycaemia after human islet transplantation into mouse liver," *Diabetologia*, vol. 53, no. 8, pp. 1669–1679, 2010.
- [241] T. Kheradmand, S. Wang, R. F. Gibly et al., "Permanent protection of PLG scaffold transplanted allogeneic islet grafts in diabetic mice treated with ECDCI-fixed donor splenocyte infusions," *Biomaterials*, vol. 32, no. 20, pp. 4517–4524, 2011.
- [242] V. Vaithilingam, J. Oberholzer, G. J. Guillemin, and B. E. Tuch, "Beneficial effects of desferrioxamine on encapsulated human islets-in vitro and in vivo study," *American Journal of Transplantation*, vol. 10, no. 9, pp. 1961–1969, 2010.
- [243] A. Langlois, W. Bietiger, K. Mandes et al., "Overexpression of vascular endothelial growth factor in vitro using deferrioxamine: a new drug to increase islet vascularization during transplantation," *Transplantation Proceedings*, vol. 40, no. 2, pp. 473–476, 2008.
- [244] A. Langlois, W. Bietiger, M. C. Sencier et al., "Adenoviral infection or deferrioxamine? Two approaches to overexpress VEGF in β -cell lines," *Journal of Drug Targeting*, vol. 17, no. 6, pp. 415–422, 2009.
- [245] A. Langlois, W. Bietiger, E. Seyfritz et al., "Improvement of rat islet viability during transplantation: validation of pharmacological approach to induce VEGF overexpression," *Cell Transplantation*. In press.
- [246] V. Vaithilingam, B. Barbaro, J. Oberholzer, and B. E. Tuch, "Functional capacity of human islets after long-distance shipment and encapsulation," *Pancreas*, vol. 40, no. 2, pp. 247–252, 2011.
- [247] M. Qi, B. L. Strand, Y. Mørch et al., "Encapsulation of human islets in novel inhomogeneous alginate- Ca^{2+} / Ba^{2+} microbeads: in vitro and in vivo function," *Artificial Cells, Blood Substitutes, and Biotechnology*, vol. 36, no. 5, pp. 403–420, 2008.
- [248] A. G. Mallett and G. S. Korbutt, "Alginate modification improves long-term survival and function of transplanted encapsulated islets," *Tissue Engineering A*, vol. 15, no. 6, pp. 1301–1309, 2009.
- [249] G. Langlois, J. Dusseault, S. Bilodeau, S. K. Tam, D. Magassouba, and J. P. Hallé, "Direct effect of alginate purification on the survival of islets immobilized in alginate-based microcapsules," *Acta Biomaterialia*, vol. 5, no. 9, pp. 3433–3440, 2009.
- [250] G. S. Korbutt, A. G. Mallett, Z. Ao, M. Flashner, and R. V. Rajotte, "Improved survival of microencapsulated islets during in vitro culture and enhanced metabolic function following transplantation," *Diabetologia*, vol. 47, no. 10, pp. 1810–1818, 2004.
- [251] T. Ikemoto, S. Matsumoto, T. Itoh et al., "Assessment of islet quality following international shipping of more than 10,000 km," *Cell Transplantation*, vol. 19, no. 6-7, pp. 731–741, 2010.
- [252] H. Ichii, Y. Sakuma, A. Pileggi et al., "Shipment of human islets for transplantation," *American Journal of Transplantation*, vol. 7, no. 4, pp. 1010–1020, 2007.
- [253] P. R. Rozak, B. P. Weegman, E. S. Avgoustiniatos et al., "Devices and methods for maintenance of temperature and pressure during islet shipment," *Transplantation Proceedings*, vol. 40, no. 2, pp. 407–410, 2008.

- [254] A. Plesner, P. Liston, R. Tan, R. G. Korneluk, and C. B. Verchere, "The X-linked inhibitor of apoptosis protein enhances survival of murine islet allografts," *Diabetes*, vol. 54, no. 9, pp. 2533–2540, 2005.
- [255] J. A. Emamaullee and A. M. J. Shapiro, "Interventional strategies to prevent β -cell apoptosis in islet transplantation," *Diabetes*, vol. 55, no. 7, pp. 1907–1914, 2006.
- [256] J. A. Emamaullee, R. V. Rajotte, P. Liston et al., "XIAP overexpression in human islets prevents early posttransplant apoptosis and reduces the islet mass needed to treat diabetes," *Diabetes*, vol. 54, no. 9, pp. 2541–2548, 2005.
- [257] J. A. Emamaullee, L. Stanton, C. Schur, and A. M. J. Shapiro, "Caspase inhibitor therapy enhances marginal mass islet graft survival and preserves long-term function in islet transplantation," *Diabetes*, vol. 56, no. 5, pp. 1289–1298, 2007.
- [258] J. A. Emamaullee, J. Davis, R. Pawlick et al., "The caspase selective inhibitor EP1013 augments human islet graft function and longevity in marginal mass islet transplantation in mice," *Diabetes*, vol. 57, no. 6, pp. 1556–1566, 2008.
- [259] A. D. Kirk, D. M. Harlan, N. N. Armstrong et al., "CTLA4-Ig and anti-CD40 ligand prevent renal allograft rejection in primates," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 16, pp. 8789–8794, 1997.
- [260] J. A. Emamaullee, J. Davis, R. Pawlick et al., "Caspase inhibitor therapy synergizes with costimulation blockade to promote indefinite islet allograft survival," *Diabetes*, vol. 59, no. 6, pp. 1469–1477, 2010.
- [261] N. Fiaschi-Taesch, A. F. Stewart, and A. Garcia-Oc a, "Improving islet transplantation by gene delivery of hepatocyte growth factor (HGF) and its downstream target, protein kinase B (PKB)/Akt," *Cell Biochemistry and Biophysics*, vol. 48, no. 2-3, pp. 191–199, 2007.
- [262] A. S. Narang, O. Sabek, A. O. Gaber, and R. I. Mahato, "Co-expression of vascular endothelial growth factor and interleukin-1 receptor antagonist improves human islet survival and function," *Pharmaceutical Research*, vol. 23, no. 9, pp. 1970–1982, 2006.
- [263] D. Jir k, J. Kriz, V. Herynek et al., "MRI of transplanted pancreatic islets," *Magnetic Resonance in Medicine*, vol. 52, no. 6, pp. 1228–1233, 2004.
- [264] P. Reimer and T. Balzer, "Ferucarbotran (Resovist): a new clinically approved RES-specific contrast agent for contrast-enhanced MRI of the liver: properties, clinical development, and applications," *European Radiology*, vol. 13, no. 6, pp. 1266–1276, 2003.
- [265] Z. Berkova, D. Jirak, K. Zacharovova et al., "Labeling of pancreatic islets with iron oxide nanoparticles for in vivo detection with magnetic resonance," *Transplantation*, vol. 85, no. 1, pp. 155–159, 2008.
- [266] Z. Berkova, J. Kriz, P. Girman et al., "Vitality of pancreatic islets labeled for magnetic resonance imaging with iron particles," *Transplantation Proceedings*, vol. 37, no. 8, pp. 3496–3498, 2005.
- [267] C. Toso, J. P. Vallee, P. Morel et al., "Clinical magnetic resonance imaging of pancreatic islet grafts after iron nanoparticle labeling," *American Journal of Transplantation*, vol. 8, no. 3, pp. 701–706, 2008.
- [268] H. S. Kim, Y. Choi, I. C. Song, and W. K. Moon, "Magnetic resonance imaging and biological properties of pancreatic islets labeled with iron oxide nanoparticles," *NMR in Bio-medicine*, vol. 22, no. 8, pp. 852–856, 2009.
- [269] F. Saudek, D. Jir k, P. Girman et al., "Magnetic resonance imaging of pancreatic islets transplanted into the liver in humans," *Transplantation*, vol. 90, no. 12, pp. 1602–1606, 2010.
- [270] D. Jirak, J. Kriz, M. Strzelecki et al., "Monitoring the survival of islet transplants by MRI using a novel technique for their automated detection and quantification," *Magnetic Resonance Materials in Physics, Biology and Medicine*, vol. 22, no. 4, pp. 257–265, 2009.
- [271] M. L. Malosio, A. Esposito, A. Poletti et al., "Improving the procedure for detection of intrahepatic transplanted islets by magnetic resonance imaging," *American Journal of Transplantation*, vol. 9, no. 10, pp. 2372–2382, 2009.
- [272] F. Ris, M. Lepetit-Coiffe, P. Meda et al., "Assessment of human islet labeling with clinical grade iron nanoparticles prior to transplantation for graft monitoring by MRI," *Cell Transplantation*, vol. 19, no. 12, pp. 1573–1585, 2010.
- [273] J. H. Juang, J. J. Wang, C. R. Shen et al., "Magnetic resonance imaging of transplanted mouse islets labeled with chitosan-coated superparamagnetic iron oxide nanoparticles," *Transplantation Proceedings*, vol. 42, no. 6, pp. 2104–2108, 2010.
- [274] J. H. Juang, C. R. Shen, J. J. Wang et al., "Magnetic resonance imaging study of mouse islet allotransplantation," *Transplantation Proceedings*, vol. 42, no. 10, pp. 4217–4220, 2010.
- [275] L. Leoni, S. D. Serai, R. L. Magin, and B. B. Roman, "Functional MRI characterization of isolated human islet activation," *NMR in Biomedicine*, vol. 23, no. 10, pp. 1158–1165, 2010.
- [276] Y. Lu, H. Dang, B. Middleton et al., "Bioluminescent monitoring of islet graft survival after transplantation," *Molecular Therapy*, vol. 9, no. 3, pp. 428–435, 2004.
- [277] X. Chen, X. Zhang, C. S. Larson, M. S. Baker, and D. B. Kaufman, "In vivo bioluminescence imaging of transplanted islets and early detection of graft rejection," *Transplantation*, vol. 81, no. 10, pp. 1421–1427, 2006.
- [278] X. Chen and D. B. Kaufman, "Bioluminescent imaging of transplanted islets," *Methods in Molecular Biology*, vol. 574, pp. 75–85, 2009.
- [279] J. Virostko, A. Radhika, G. Poffenberger et al., "Bioluminescence imaging in mouse models quantifies β cell mass in the pancreas and after islet transplantation," *Molecular Imaging and Biology*, vol. 12, no. 1, pp. 42–53, 2010.
- [280] E. J. Grossman, D. D. Lee, J. Tao et al., "Glycemic control promotes pancreatic beta-cell regeneration in streptozotocin-induced diabetic mice," *PLoS ONE*, vol. 5, no. 1, Article ID e8749, 2010.
- [281] E. Grossman, D. Lee, J. Tao et al., "Seeing is believing: how the MIP-luc mouse can advance the field of islet transplantation and β -cell regeneration," *Islets*, vol. 2, no. 4, pp. 261–262, 2010.
- [282] C. E. Morrow, J. I. Cohen, D. E. R. Sutherland, and J. S. Najarian, "Chronic pancreatitis: long-term surgical results of pancreatic duct drainage, pancreatic resection, and near-total pancreatectomy and islet autotransplantation," *Surgery*, vol. 96, no. 4, pp. 608–616, 1984.
- [283] D. E. R. Sutherland, A. J. Matas, F. C. Goetz, and J. S. Najarian, "Transplantation of dispersed pancreatic islet tissue in humans. Autografts and allografts," *Diabetes*, vol. 29, supplement 1, pp. 31–44, 1980.
- [284] T. Kobayashi, J. C. Manivel, A. M. Carlson et al., "Correlation of histopathology, islet yield, and islet graft function after islet autotransplantation in chronic pancreatitis," *Pancreas*, vol. 40, no. 2, pp. 193–199, 2011.
- [285] M. Takita, B. Naziruddin, S. Matsumoto et al., "Variables associated with islet yield in autologous islet cell transplantation for chronic pancreatitis," *Proceedings of Baylor University Medical Center*, vol. 23, no. 2, pp. 115–120, 2010.

- [286] J. L. Argo, J. L. Contreras, M. M. Wesley, and J. D. Christein, "Pancreatic resection with islet cell autotransplant for the treatment of severe chronic pancreatitis," *American Surgeon*, vol. 74, no. 6, pp. 530–536, 2008.
- [287] J. J. Blondet, A. M. Carlson, T. Kobayashi et al., "The role of total pancreatectomy and islet autotransplantation for chronic pancreatitis," *Surgical Clinics of North America*, vol. 87, no. 6, pp. 1477–1501, 2007.
- [288] S. A. Ahmad, A. M. Lowy, C. J. Wray et al., "Factors associated with insulin and narcotic independence after islet autotransplantation in patients with severe chronic pancreatitis," *Journal of the American College of Surgeons*, vol. 201, no. 5, pp. 680–687, 2005.
- [289] A. C. Farney, J. S. Najarian, R. E. Nakhleh et al., "Autotransplantation of dispersed pancreatic islet tissue combined with total or near-total pancreatectomy for treatment of chronic pancreatitis," *Surgery*, vol. 110, no. 2, pp. 427–439, 1991.
- [290] H. L. Rodriguez Rilo, S. A. Ahmad, D. D'Alessio et al., "Total pancreatectomy and autologous islet cell transplantation as a means to treat severe chronic pancreatitis," *Journal of Gastrointestinal Surgery*, vol. 7, no. 8, pp. 978–989, 2003.
- [291] G. Garcea, J. Weaver, J. Phillips et al., "Total pancreatectomy with and without islet cell transplantation for chronic pancreatitis: a series of 85 consecutive patients," *Pancreas*, vol. 38, no. 1, pp. 1–7, 2009.
- [292] M. D. Bellin, D. E. Sutherland, G. J. Beilman et al., "Pancreatectomy and autologous islet transplantation: a study of long-term outcomes," in *Pancreas Club Annual Meeting Program Book*, vol. 21, 2007.
- [293] M. A. Webb, S. C. Illouz, C. A. Pollard et al., "Islet auto transplantation following total pancreatectomy: a long-term assessment of graft function," *Pancreas*, vol. 37, no. 3, pp. 282–287, 2008.
- [294] S. Illouz, M. Webb, C. Pollard et al., "Islet autotransplantation restores normal glucose tolerance in a patient with chronic pancreatitis," *Diabetes Care*, vol. 30, no. 12, article e130, 2007.
- [295] H. A. Clayton, J. E. Davies, C. A. Pollard, S. A. White, P. P. Musto, and A. R. Dennison, "Pancreatectomy with islet autotransplantation for the treatment of severe chronic pancreatitis: the first 40 patients at the Leicester General Hospital," *Transplantation*, vol. 76, no. 1, pp. 92–98, 2003.
- [296] D. E. R. Sutherland, A. C. Gruessner, A. M. Carlson et al., "Islet autotransplant outcomes after total pancreatectomy: a contrast to islet allograft outcomes," *Transplantation*, vol. 86, no. 12, pp. 1799–1802, 2008.
- [297] J. J. Blondet, A. M. Carlson, T. Kobayashi et al., "The role of total pancreatectomy and islet autotransplantation for chronic pancreatitis," *Surgical Clinics of North America*, vol. 87, no. 6, pp. 1477–1501, 2007.
- [298] M. D. Bellin, A. M. Carlson, T. Kobayashi et al., "Outcome after pancreatectomy and islet autotransplantation in a pediatric population," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 47, no. 1, pp. 37–44, 2008.
- [299] M. D. Bellin and D. E. R. Sutherland, "Pediatric islet auto-transplantation: indication, technique, and outcome," *Current Diabetes Reports*, vol. 10, no. 5, pp. 326–331, 2010.
- [300] D. C. Wahoff, B. E. Papalois, J. S. Najarian et al., "Islet auto-transplantation after total pancreatectomy in a child," *Journal of Pediatric Surgery*, vol. 31, no. 1, pp. 132–136, 1996.
- [301] M. D. Bellin, J. J. Blondet, G. J. Beilman et al., "Predicting islet yield in pediatric patients undergoing pancreatectomy and autoislet transplantation for chronic pancreatitis," *Pediatric Diabetes*, vol. 11, no. 4, pp. 227–234, 2010.
- [302] T. Kobayashi, J. C. Manivel, M. D. Bellin et al., "Correlation of pancreatic histopathologic findings and islet yield in children with chronic pancreatitis undergoing total pancreatectomy and islet autotransplantation," *Pancreas*, vol. 39, no. 1, pp. 57–63, 2010.

Review Article

Molecular Imaging: A Promising Tool to Monitor Islet Transplantation

Ping Wang, Zdravka Medarova, and Anna Moore

Molecular Imaging Laboratory, MGH/MIT/HMS Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, 02129, USA

Correspondence should be addressed to Anna Moore, amoore@helix.mgh.harvard.edu

Received 31 May 2011; Accepted 29 July 2011

Academic Editor: Antonello Pileggi

Copyright © 2011 Ping Wang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Replacement of insulin production by pancreatic islet transplantation has great potential as a therapy for type 1 diabetes mellitus. At present, the lack of an effective approach to islet grafts assessment limits the success of this treatment. The development of molecular imaging techniques has the potential to fulfill the goal of real-time noninvasive monitoring of the functional status and viability of the islet grafts. We review the application of a variety of imaging modalities for detecting endogenous and transplanted beta-cell mass. The review also explores the various molecular imaging strategies for assessing islet delivery, the metabolic effects on the islet grafts as well as detection of immunorejection. Here, we highlight the use of combined imaging and therapeutic interventions in islet transplantation and the in vivo monitoring of stem cells differentiation into insulin-producing cells.

1. Introduction

Type 1 diabetes mellitus (T1DM) is characterized by an absolute deficiency of insulin secretion with hyperglycemia as a consequence. T1DM is one of the most common diseases of childhood. 13,000 new cases are diagnosed each year in North America [1]. The first major breakthrough in the treatment of T1DM was the isolation of insulin and use of its synthetic forms. Although insulin has changed the clinical course of T1DM from an acutely fatal disease to a chronic one with severe long-term complications, it does not cure diabetes [2]. In 1966, the first whole pancreas transplantation was performed [3]. Clinical studies show that pancreatic allotransplantation offers superior glycemic control for T1DM and prevents or even reverses secondary complications, including nephropathy [4]. The elevated risk of surgical complications and the relative invasiveness of the procedure, however, makes the practice of solid organ transplantation rare in T1DM patients. Since 1999, when the first accounts of consistent success in restoring normoglycemia using islet transplantation appeared, this less invasive procedure has become an important alternative treatment for T1DM patients.

The first attempt to transplant an islet cell xenograft was performed in 1893, 29 years before the isolation of insulin. A 15-year-old diabetic patient was transplanted sheep pancreatic tissue beneath his skin. The procedure failed and the patient died after 3 days [5]. In 1967, a method of isolating islets using collagenase was described [6]. This launched the earliest islet transplantation in animal models in 1972 [7]. The first clinical islet allograft was performed in 1974 [8]. The next 25 years have witnessed attempts to achieve normoglycemia in type 1 diabetic patients using islet transplantation, with limited success. In 1999, the Edmonton protocol revived interest in this procedure by reporting reproducible success in terms of insulin independence through islet transplantation [9]. All of the patients maintained insulin independence after 1 year of followup [10]. This new protocol relies on a prednisone-free immunosuppressive regimen and improved islet delivery through intraportal infusion of freshly isolated islets, followed by a second or third infusion of additional islets [8]. With this protocol, from 1999 to 2008, almost 400 patients received allogeneic islet transplants [11]. However, with more follow-ups, it became apparent that insulin independence was only transient in most recipients. A recent international trial using

the Edmonton protocol showed that only 44% of the patients receiving islet transplantation remained insulin independent at one year after-transplant [12]. Unfortunately, only less than 10% of the recipients remain insulin independent for up to 5 years [13].

It is believed that many factors contribute to the loss of graft function. Early losses are primarily linked to damage sustained during the isolation procedure or in the graft microenvironments, secondary to ischemia-reperfusion-like injury and nonspecific inflammation such as the instant blood-mediated inflammatory reaction (IBMIR) [14]. Subsequent losses are usually more progressive and involve several immunological related factors. The presence of allogeneic rejection has been strongly suggested by the poorer clinical outcomes in case of prior human leukocyte antigen sensitization and, conversely, by favorable results achieved with more potent immunosuppression treatment [15]. The recurrence of autoimmunity also is a major limiting factor in long-term survival of islet grafts. Some studies have shown that monocytic infiltration of the graft occurs as early as 14 days after transplantation, with a preferential loss of insulin-secreting beta cells [16]. After islet transplantation, elevated islet cell autoantibody titers (to glutamic acid decarboxylase or GAD65) persisted [17]. Immunosuppressive drug toxicity is another immune-related assault on the graft. It has been demonstrated that rapamycin, at a concentration usually used to prevent islet grafts rejection, is able to reduce the rate of beta cell proliferation not only in transplanted rat islets but also in host murine islets, suggesting that the progressive islet grafts dysfunction observed under immunosuppressive therapy may result in part from an impairment in beta cells regeneration [18].

It is clear by now that an effective approach to islet grafts assessment following transplantation is urgently needed. Successful monitoring of the graft would allow us to test the viability and functionality of the graft. Such monitoring would also provide a better understanding of the various mechanisms involved in graft loss. It would also permit us to design and implement prompt intervention and more carefully tailored treatment. However, currently the majority of methods for assessment of the islet grafts are still indirect. These include indicators of metabolic control, such as fasting and stimulated glucose levels, oral glucose tolerance testing (OGTT), C-peptide levels, HbA1c levels, mean amplitude glycemic excursions (MAGE), and insulin secretion. In addition, immune events and complications associated with islet transplantation are indirectly tested as well. These include allo- and autoimmune antibodies as well as signs of toxicity or impairment of liver function [19]. All of these indirect parameters only provide information on the late stages of graft rejection. Since the mechanisms behind islet function represent a finelytuned network of regulated interactions and feedback loops, alterations in C-peptide and insulin release do not become apparent until most islets have already been destroyed [20–22]. The only direct morphological assessment of transplant fate in the clinic is obtained through histological biopsy. However, it could not be widely applied due to the small size of islet grafts and their relatively low frequency dispersed in a large

organ such as the liver. Moreover, this approach is invasive. Therefore, it is critical to establish a noninvasive method to monitor the fate of islets directly in a clinical setting.

Molecular imaging is a rapidly emerging biomedical research discipline. Considerable efforts have been directed in recent years toward the development of noninvasive high-resolution *in vivo* imaging technologies including optical imaging, nuclear imaging, and magnetic resonance imaging (MRI). At the same time, various molecular imaging probes with greater specificity and targeting potential have been designed and tested (antibodies, ligands, or substrates that can specifically interact with targets in particular cells or sub-cellular compartments) [23]. The development of molecular imaging techniques has the potential to fulfill the goal of real-time non-invasive monitoring of the functional status and viability of the islet grafts after transplantation. The present paper explores the various preclinical and clinical molecular imaging strategies for the tracking of graft fate, islet delivery strategies, as well as detection of immunorejection. We also review the use of combined imaging and therapeutic approaches in islet transplantation [24] and the *in vivo* monitoring of embryonic stem cells differentiation into insulin-producing cells.

2. Imaging Beta Cells as a Key Step towards Understanding Islet Transplantation

Progress towards the goal of direct assessment of graft integrity, viability, and function would rest on previous experience in the area of pancreatic islet imaging as a tool for measurement of islet mass and integrity. Imaging beta cells represents a daunting challenge both from a biological and a technological perspective, owing to the innately complex structure and distribution of pancreatic islets within the pancreas and the elaborate dynamic nature of their metabolic function. Pancreatic islets are small structures (100–400 μm in diameter) that are dispersed throughout the pancreas and constitute only 2% of the pancreatic volume [25]. A variety of imaging modalities have shown promise exclusively in animal models.

2.1. Bioluminescence and Fluorescence Optical Imaging. In 2003, Hara et al. [26] generated transgenic mice that express green fluorescent protein (GFP) under the control of the mouse insulin I gene promoter (MIP). Histological studies showed that the MIP-GFP mice had normal islet architecture with coexpression of insulin and GFP in islet beta cells. GFP-expressing beta cells could be then imaged *in vivo* allowing for beta cell mass determination. Our group described the *ex vivo* imaging of beta-cell apoptosis with a near-infrared (NIR) probe (Cy5.5-labeled annexin V) in 2005 [27]. Following, we described the synthesis and testing of an NIR probe for imaging beta-cells in pancreatic islets, which was based on the beta-cells-specific ligand streptozotocin (STZ) labeled with Cy5.5. We observed a bright fluorescence signal consistent with intracellular accumulation of the probe, which was mediated by the glucose transporter 2 (GLUT2 transporter) [28]. Another recent example of a

fluorescent probe for detecting beta cells is a near-infrared fluorescent exendin-4 analogue with specificity for the Glucagon-like peptide 1 (GLP-1) receptor on beta cells. Following intravenous administration into mice, pancreatic islets were readily distinguishable from exocrine pancreas, achieving target-to-background ratios within the pancreas of 6:1 using intravital microscopy [29]. Bioluminescence imaging of beta-cell mass was applied by Virostko et al. [30] in a recent study where a transgenic mouse model expressing luciferase under control of the mouse insulin I promoter (mouse insulin promoter-luciferase-Vanderbilt University (MIP-Luc-VU)) was used. This model enabled non-invasive assessment of changes in beta-cell mass after islet transplantation based on changes in bioluminescence signal.

2.2. Nuclear Imaging. An attractive approach to image beta cell is nuclear imaging. The major advantage of nuclear imaging is its high sensitivity. It relies on radionuclide-labeled contrast agents that target beta cells based on cell-specific antigens, receptors, metabolites, or pharmacologic agents.

Nuclear imaging has been utilized by our group to estimate beta-cell mass using an ^{111}In -labeled monoclonal antibody targeting the beta-cell surface antigen IC2 [31]. In this work ^{111}In -labeled IC2 was characterized *in vitro* in islets as well as *in vivo* in a diabetic mouse model. Other examples of agents that target beta cells directly through surface antigens include ^{125}I -labelled monoclonal antibody R2D6 directed against gangliosides on the plasma membranes of pancreatic beta cells [32] as well as phage-display-derived peptides [33]. In order to overcome some of the weaknesses of monoclonal antibodies as radiotracers, single-chain antibodies (SCAs) were developed. It was reported that removal of the Fc portion to produce an antibody fragment reduced the nonspecific binding to beta cells [34, 35].

Extensive nuclear imaging studies have been performed targeting receptors expressed on beta-cell surface. The vesicular monoamine transporter 2 (VMAT2) is a monoamine transporting integral membrane protein expressed by rodent and human beta cells [36]. Tetrabenazine (TBZ) and Dihydro-tetrabenazine (DTBZ) specifically bind to the synaptic VMAT2 [37]. A clinical study showed a reduction in pancreatic uptake of ^{11}C -DTBZ in long-standing T1D patients compared to the uptake in the pancreas of healthy control subjects [38]. DTBZ compounds labeled with ^{18}F were developed to overcome the short half-life of ^{11}C -labeled DTBZ ($T_{1/2} = 20$ min). Preclinical studies showed high pancreatic uptake of ^{18}F -DTBZ in healthy rats with favorable biodistribution leading to improved target-to-background ratios [39]. Recently another new DTBZ derivative, ^{18}F -FP-(+)-DTBZ, was tested. The result showed that this compound had higher pancreatic uptake and lower uptake in the nontarget tissues, especially the liver [40]. The glucagon-like peptide-1 (GLP-1) receptor is a potential target for beta cells imaging as well. It is triggered after binding of the agonists Exendin-3 and Exendin-4 [41]. The first preclinical study using ^{123}I -labeled Exendin showed high uptake in the pancreas and in subcutaneous insulinomas [42]. Furthermore,

it has been shown that the uptake of ^{111}In -DTPA-Lys40-Exendin-3 correlates with beta-cell mass in a linear manner in diabetic rats and that the pancreas can be visualized by SPECT imaging on a dedicated microSPECT scanner [43, 44]. Radiolabeled pharmacologic agents targeting other receptors also have been investigated. They include ligands to sulfonylurea receptors, such as glyburide or tolbutamide analogs [45–47]. ^{18}F -L-DOPA [48] targeting dopamine receptor may also be a suitable approach for the detection of beta-cells.

Various tracers based on radiolabeled glucose have also been explored for targeting beta cells and, of those, the most promising has proven to be mannoheptulose and tritiated D-mannoheptulose, which is apparently transported into cells mainly at the intervention of GLUT-2 [49–51].

2.3. MR Imaging. One of the most logical modalities to explore for imaging of pancreatic islets is MRI. MRI does not utilize ionizing radiation, has tomographic capabilities, can deliver the highest-resolution images *in vivo*, and has unlimited depth penetration. MR imaging has an overall low sensitivity in detecting molecular probes (10^{-3} – 10^{-5} M) [23]. However, this drawback can be overcome by the application of contrast agents that amplify the signal. New agents such as a novel class of lanthanide complexes for labeling beta cells were first reported at the 2003 NIH Workshop on Imaging Pancreatic Beta cells [52]. ^1H NMR spectroscopy has been suggested for measuring choline levels, which can possibly indicate the number of viable cells in islet grafts [53]. One group's studies demonstrated that *in vitro* ^1H NMR imaging could be used to visualize islets or βTC3 cells within their encapsulated environment. They also showed that localizing implanted microencapsulation-based bioartificial pancreas *in vivo* was feasible with the use of diffusion-weighted imaging [54]. ^{13}C spectroscopy has also been applied for studying glucose-stimulated insulin secretion and has shown promise for the investigation of beta-cell function [55]. Another interesting approach demonstrated the feasibility of direct imaging of beta-cell activation in the presence of divalent manganese cations Mn^{2+} [56, 57]. Recently, Mn^{2+} -enhanced MRI was applied for noninvasive detection of beta cell function after glucose infusion. Serial inversion recovery MRI was subsequently performed to probe for Mn^{2+} accumulation in pancreas. This experiment demonstrated the potential of Mn^{2+} -enhanced MRI for noninvasive monitoring of beta cell function [58].

A multimodal approach for imaging beta cells was applied by Yong et al. [59]. Transgenic MIP-TF C57/BL6 mice were generated in which beta-cells express a fusion of three different imaging reporters. Multimodal imaging of MIP-TF pancreatic beta-cells was demonstrated by fluorescence microscopy, BLI, and microPET. The MIP-TF mice enabled noninvasive monitoring of beta-cells in models of type 1 and type 2 diabetes. This multimodality imaging animal model might expedite studies in a broad range of diabetes research.

Despite these encouraging examples, however, in the authors' opinion, the issue of visualizing beta-cell mass *in vivo* using noninvasive imaging remains an unsolved challenge.

The ultimate solution to this problem would likely require the identification of specific beta-cell markers and targeting ligands, as well as improvements in imaging technology to permit the acquisition of quantitative information with both high sensitivity and high spatial resolution. The latter would probably rely on the development of multimodality approaches and would demand sophisticated image analysis tools to monitor even small changes in the signal reflective of the dynamic nature of beta-cell mass.

3. Imaging of Transplanted Islets—Current Progress

The possibility of directly imaging transplanted islets rests on the fact that isolated islets can be labeled before-transplant using various approaches, including genetic modification with fluorescent or bioluminescent reporters, labeling with exogenous contrast agents, such as superparamagnetic iron oxides for MRI or radiolabeled metabolites for nuclear imaging. These general strategies for the monitoring of transplanted islets by noninvasive imaging have proven valuable in answering questions about graft fate, designing new therapeutic interventions, and testing alternative transplant sites, all ultimately aimed at extending graft longevity and function.

Here, we highlight some of the progress made using different imaging modalities in acquiring new knowledge about islet transplantation. These studies represent just the first steps towards realizing the true potential of noninvasive imaging to tackle biological questions. The unique advantage of noninvasive imaging lies in its capacity to provide information in authentic physiologic environments, in real-time, and on a systemic level.

3.1. Bioluminescence and Fluorescence Optical Imaging. The first evidence of the feasibility of imaging transplanted islets noninvasively was obtained using bioluminescence imaging (BLI). Several laboratories [60–62] reported proof-of-principle studies in which isolated rodent or human islets were genetically engineered to express luciferase and imaged following transplantation. In islets transplanted underneath the renal capsule of immunocompromised mice, the magnitude of the signal was dependent on the islet dose, indicating that the collected information could be used to obtain accurate quantitative information about islet number over time. Although adenovirus-directed luciferase expression attenuated, consistent with the transient nature of the vector, lentivirus vectors could be used to direct the long-term expression of reporter genes in transduced islets. Furthermore, the functionality of transduced islets was retained since transplanted lentivirus-transduced islets led to long term restoration of euglycemia in diabetic mice. Finally, these studies provided new information about islet fate following transplantation. Luciferase signal emanating from the graft remained stable for at least 140 days, indicating graft stability in this model of transplantation [62]. The feasibility of monitoring transplanted islets by BLI was also demonstrated in the intrahepatic transplantation model. Long-term monitoring

of adenovirus-transduced islets transplanted into the livers of immunocompromised mice, suggested that, in the described animal model, the intrahepatic islet grafts were also stable, [63]. Early detection of graft rejection was also possible using BLI [64, 65].

The first study of the fluorescence optical imaging of islet grafts was published in 2003 [66]. In this study, the graft was monitored with a fused fluorescent reporter protein. In 2006, our group reported that islets incubated with nanoparticle probes (labeled with near-infrared fluorescent Cy5.5 dye) could be detected in the near-infrared channel under the kidney capsule in vivo after transplantation [67].

Despite the lack of a clinical equivalent of these modalities, the information gained from BLI and fluorescence imaging represents a variable contribution to the field of islet transplantation. Therefore, these studies demonstrated the feasibility of conducting preclinical research in small animals in order to address some basic questions about transplanted islet biology and begin to develop alternative strategies for the enhancement of graft function.

3.2. Nuclear Imaging. Unlike bioluminescence imaging, nuclear imaging has a clinical equivalent and therefore is more likely to evolve into a modality routinely used in hospitals for monitoring of patients that receive islet transplantation therapy. Initial reports regarding the application of radionuclide imaging to monitor transplanted pancreatic islet grafts employed islets genetically engineered to express a mutant herpes simplex virus type 1 thymidine kinase (sr39tk). The expressed enzyme can phosphorylate positron-emitting, radionuclide-labeled thymidine analogues or acycloguanosine substrates once they are delivered inside the cell, leading to trapping of the phosphorylated product and its detection by positron emission tomography. In one of the early studies, islets were transduced with an Adeno-Tkm adenovirus engineered to express sr39tk under a constitutive promoter [69]. Mice were subjected to microPET imaging after injection of the sr39tk substrate [¹⁸F] FHBG. As observed by BLI [62], the signal was unstable after a few weeks, likely due to the transient expression of adenovirally directed reporter genes. By contrast, lentiviral transduction of pancreatic islets with sr39tk was used for the long-term monitoring of transplanted islet fate [70]. Islets implanted in the liver were detectable for several weeks after transplantation, suggesting the persistence of the graft. A similar approach was used to monitor the expression of a therapeutic gene (interleukin-10), which could prolong the survival of islet grafts under the kidney capsule of diabetic mice [71]. Although very valuable from a research perspective the reporter nuclear imaging studies are not likely to apply in a clinical scenario in the near future since they involve genetic modification of the islets before-transplant.

A more clinically relevant technique for the PET imaging of early posttransplant events involved labeling of rodent islets with 2-¹⁸F] fluoro-2-deoxy-D-glucose (FDG) and their subsequent implantation in the livers of syngeneic rats. The grafts were detected for up to 6 hours [72]. This model, however, is only useful for the short-term monitoring of

transplanted islet fate, since the persistence of the label in the islet cells is unknown. Furthermore, the short half-life of ^{18}F (110 min) adds to the difficulty of long-term graft monitoring. Without a clear understanding of how long the islets retain the label if at all, it is difficult to obtain quantitative information about islet abundance through time, using this method. In subsequent studies performed in large animals, only ~50% of the administered radioactivity was observed in the liver at the end of islet infusion, likely due to islet damage or FDG leakage from the islets [73]. Nevertheless, the clinical feasibility of detecting transplanted islets labeled with [^{18}F] FDG was demonstrated for the first time in 2007 [74]. The same group reported that islets labeled with [^{18}F] FDG transplanted to 6 patients could be detected during the first 1-2 h. Beyond this, the radioactive half-life and retention within the islets limit the use of this method [68]. Recently, clinical testing of GLP-1 receptor was applied for imaging of human beta cells transplanted in patient muscle, which showed its potential to assess islet survival in clinical transplantation [44]. However, long-term monitoring after-transplant presents a significant problem associated with this method.

3.3. Magnetic Resonance Imaging. Magnetic resonance imaging (MRI) is a modality, which, like nuclear imaging, has a broad clinical applicability and can be used to monitor transplanted pancreatic islets. Whereas nuclear imaging is characterized by high sensitivity to contrast agent abundance and a highly quantitative correlation between signal and local concentration of contrast agent, MRI is tomographic and can acquire images with a high spatial resolution. Therefore, MRI, unlike nuclear imaging, can be used to study directly the abundance and tissue distribution of transplanted islets.

The innate low sensitivity of MRI can be overcome with the use of contrast agents, such as superparamagnetic iron oxide nanoparticles. Superparamagnetic iron oxide nanoparticles have been extensively used as magnetic resonance reporters. Their basic structure includes an iron oxide core covered with a dextran coat [75] that can be functionalized with additional imaging, targeting, or therapeutic moieties. The presence of iron oxides in tissue is evidenced by a loss in signal intensity on T2-weighted and T2*-weighted MR images or, in technical terms, by a shortening of the T2 relaxation time of surrounding water protons.

Several groups focused on superparamagnetic iron oxides [67, 76, 77] for the labeling of isolated pancreatic islets before-transplant, followed by their noninvasive monitoring using MRI. In the study performed in our laboratory, isolated human pancreatic islets were labeled with superparamagnetic iron oxide magnetic nanoparticles (MNs) modified with a near-infrared fluorescent dye (MN-NIRF) and transplanted under the kidney capsule in immunocompromised mice. MN-NIRF-labeled human pancreatic islets were visualized for up to 188 days after transplantation in this model demonstrating graft stability and persistence of the label (Figure 1). The labeled islets were viable and functional, since the graft could restore normoglycemia in diabetic mice [67].

In more recent experiments, we also demonstrated the applicability of the approach to the intrahepatic transplantation model. For islet labeling we utilized an FDA-approved commercially available iron oxide agent (ferumoxides), which is routinely used in the clinic for liver imaging. Similar to MN-NIRF, it consists of superparamagnetic iron oxide covered with a dextran coat. Human islets labeled with ferumoxides and transplanted into the liver appeared as distinct hypointense foci representing single islets and/or islet clusters. The persistence of the graft in immunocompromised animals was demonstrated for the entire observation period of two weeks [78].

The applicability of MRI for the visualization of transplanted islets, following their labeling with a contrast agent, was also demonstrated using paramagnetic beads [79], and a paramagnetic contrast agent, GdHPDO3A [77]. In another study, the strong relaxation effect of superparamagnetic iron oxides allowed the detection of transplanted islets at a lower magnetic field strength (1.5 T), equivalent to the ones used in hospitals today, advancing the method to a more clinically relevant stage [80].

Visualizing transplanted islet in large animal models using MRI serves as an important step before clinical application. The first study in large animals was reported in 2007 [81]. Human islets labeled with immunoprotective iron oxide-loaded magnetic capsules were detected with real-time MRI [81]. Recently, our group reported the *in vivo* imaging of autologous islet grafts in the liver and under the kidney capsule in nonhuman primates. The renal subcapsular islet grafts were easily detectable on T2*-weighted MR images as a pocket of signal loss disrupting the contour of the kidney at the transplantation site. Islets transplanted in the liver appeared as distinct signal voids dispersed throughout the liver parenchyma. This study established a method for the noninvasive, longitudinal detection of pancreatic islets transplanted into non-human primates using a low-field clinical MRI system [25].

The first imaging study applied in humans with superparamagnetic iron oxide nanoparticles was carried out in 2008 [82]. Islets were labeled with superparamagnetic iron oxide particles (SPIO, 280 microg/mL) and transplanted into patients with T1DM. All patients could stop insulin after transplantation. Three out of four patients had normal intensity on pretransplant images, and iron-loaded islets could be identified after transplantation as hypointense spots within the liver. However, this clinical study did not show any correlation between the number of labeled transplanted islets and the number of hypointense spots on MR images. In addition, the number of spots varied significantly over the course of the study making it impossible to make any conclusions regarding graft outcome. In spite of these shortcomings the study demonstrated that islet labeling is safe and islet function is not affected by labeling.

4. Imaging of Islet Grafts Rejection

4.1. Fluorescence Optical Imaging. The earliest report utilizing fluorescence optical imaging for the monitoring of

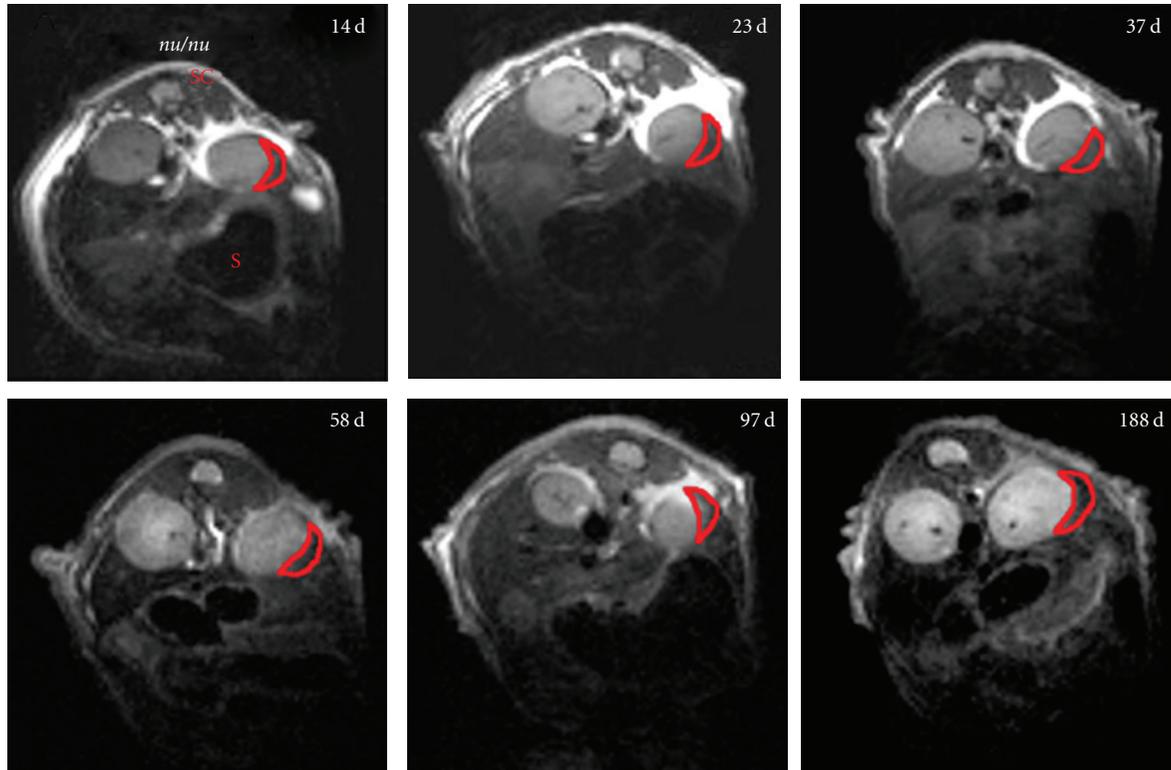


FIGURE 1: Transverse T2-weighted magnetic resonance images of transplanted labeled and nonlabeled human islets 14, 23, 37, 58, 97, and 188 d after transplantation under the kidney capsule in nude (nu/nu) mice. The dark area in the left kidney represents a labeled graft (red outline). No darkening was reported for the right kidney with unlabeled graft. S: stomach; SC: spinal cord, reproduced with permission from Nature Publishing Group [67].

immunological effects associated with islet transplantation relied on genetic engineering to create transgenic mice that express proinsulin II fused with the live-cell fluorescent reporter protein, Timer [66]. Since Timer protein changes its emission wavelength in the first 24 hr after synthesis, it can be used to monitor the time course of insulin synthesis. Islets expressing this construct were transplanted into recipient transgenic mice in which T lymphocytes were fluorescently labeled with a fluorochrome different from Timer. The animals were monitored through a body window device to derive complementary information about insulin synthesis and alloimmunity triggered by the engrafted islets. The value of this method lies in the fact that, for the first time, it allows the direct analysis not simply of islet abundance but also of islet function in the context of immune rejection.

Recently, Fan et al. have developed a new reporter mouse model, which has its T-cell expressing distinct “color-coded” proteins enabling *in vivo* detection of different T-cell subsets. With these tools, the authors found notable differences in the T cell response in islet grafts recipients receiving tolerance-inducing treatment compared to control group. These studies established real-time cell tracking tool to probe the islet grafts immunologic rejection at cellular level [83].

4.2. Bioluminescence Imaging. A comprehensive investigation into the relative effects of immune rejection on viable

islet mass was obtained by the transplantation of islets obtained from a transgenic mouse strain, which constitutively expresses firefly luciferase, underneath the renal capsule or into the liver of syngeneic or allogeneic streptozotocin-induced diabetic recipients. Whereas, in isografts, following an almost 50% decrease in signal intensity in the first 14 days after transplantation, there was an overall long-term stability of luminescence intensity signals, in allografts, graft bioluminescent intensity progressively decreased several days before the permanent recurrence of rejection-induced hyperglycemia [65]. In nontransgenic syngeneic recipients transplanted with transgenic islets, high levels of bioluminescence over the abdominal region of the liver were detected within 24 hr after [64]. Monitoring bioluminescence signal from the abdomen of the recipient for more than 90 days revealed a decline over time. With the caveat that bioluminescence signal can be influenced by a variety of factors, such as serum glucose levels, mouse positioning, and surgical and motion artifacts, and so forth, this work suggests that in the absence of immune rejection islet grafts survival can be extended significantly. Even though this conclusion is not surprising, the described method lays the groundwork for future studies in which various immunosuppression strategies can be tested for their potential to more closely emulate a syngeneic immune context.

4.3. Magnetic Resonance Imaging. Our group conducted similar studies to the ones described above in which we employed a pre-clinical model of islet transplantation at the hepatic site where islets were monitored by MRI. Immunocompetent Balb/c mice exhibited a significantly higher rate of islet loss as seen on MR images, compared to immunocompromised animals. Islet loss in the immunocompetent model was especially pronounced on day 10 after transplantation and ultimately resulted in a 20% difference in relative islet number by 14 days after transplantation (Figure 2) [78]. This report established a quantitative framework to describe the rate of islet loss in an immunocompetent context. Because of the direct comparison between the immunocompetent and immunocompromised models, this quantitative, non-invasive, and time-course sensitive imaging method allowed us to isolate out the relative contribution of allorejection to the overall decrease in transplanted islet mass in the early posttransplant period from among a multitude of factors, such as mechanical stress and vascular disruption [84].

5. Imaging of Novel Islet Transplant Sites

5.1. Bioluminescence Imaging. The clinically relevant site of islet transplantation is the liver. In research models, islets are also routinely transplanted underneath the renal capsule. Still, because of the suboptimal performance of islet grafts at these two sites, there is an ongoing effort to identify and test novel transplantation sites that provide a more suitable vascular environment for the islets, as well as a more immunoprotected physiological niche. The epididymal fat pad has emerged as an alternative transplant site, characterized by similar outcomes to intraportal transplantation. Mouse islets implanted into the intra-abdominal epididymal fat pad restored normoglycemia in STZ-treated recipients [85]. As few as 50 islets mediated similar levels of glucose tolerance when transplanted in the fat pad and the liver. When transgenic luciferase-positive islets were transplanted, bioluminescence imaging showed stability of the grafts for over 5 months. This was further supported by histological examination of the grafts showing healthy, well-granulated insulin-containing cells surrounded by healthy adipocytes [85]. These experiments suggest that the fat pad may be an alternative site of islet transplantation that is characterized by outcomes at least equivalent to the intraportal route.

5.2. Laser Scanning Microscopy (LSM). A very interesting study explored the anterior chamber of the eye as a site of islet transplantation [86]. The anterior chamber of the eye is a suitable transplantation site because it provides an immune-privileged environment and because the high amount of autonomic nerves and blood vessels found in the iris enables fast engraftment. Islets were isolated from transgenic mice expressing enhanced GFP under the control of the rat insulin-1 promoter (RIP-GFP) and used for transplantation. Simultaneous two-photon LSM (TPLSM) of beta cells (GFP signal) and the vascular network (intravenous Texas Red-conjugated 70-kDa dextran) revealed that, by day 3 after transplantation, islets attached to the iris and began to recruit

blood vessels from the iris. By day 14, blood vessels formed a microvascular network throughout the islet grafts [86, 87].

Furthermore, the authors monitored changes in cytoplasmic free Ca^{2+} concentration of the transplanted islets, as a direct indicator of islet function. Islets were loaded with the Ca^{2+} indicators Fluo-4 and Fura-Red via perfusion of the anterior chamber of the eye and imaged using LSM. Changes in cytoplasmic free Ca^{2+} concentration were successfully measured following stimulation of beta-cells activity with glibenclamide [86, 87].

Finally, the authors used LSM to noninvasively image beta cell death in islets transplanted into the anterior chamber of the eye. RIP-GFP islets were implanted into the anterior chamber of the eye and, after complete engraftment and vascularization, monitored after intravenous administration of the fluorescently labeled apoptotic marker annexin V. These experiments revealed a very low incidence of cell death in islets transplanted in this site most likely due to its immune-privileged surroundings [86, 87].

These combined studies illustrate the potential of noninvasive imaging for the collection of comprehensive information about transplanted islet biology and function on a systemic level. Islet engraftment, vascularization, function, and cell death were for the first time examined within a coherent experimental framework, providing unique knowledge on the subject of islet transplantation.

Most recently, the same group reported that they used intravital multiphoton microscopy to monitor transplanted islets in the anterior chamber of the mouse eye. This technique allowed for studies at the single-cell resolution and enabled longitudinal, noninvasive imaging of immune response within target tissues during islet allorejection [88].

5.3. Nuclear Imaging. Lu et al. [70] implanted islets adenovirally transduced with sr39tk into the axillary cavity of recipient animals. The reason the authors selected this transplantation site is because untrapped PET probe is eliminated through the gut and kidney and can create spillover background signals in the pancreas, kidney, and liver regions of small animals until it is excreted. Therefore, to avoid background signals, the authors initially tested whether sr39tk-expressing islets could be imaged after implantation into the axillary cavity, which is far from the probe elimination pathway. In this model, there was a direct relationship between microPET signal and implanted islet mass. In longitudinal studies, signal decreased by approximately one-half during the first few weeks after transplantation, followed by stabilization over 90 days, suggesting significant islet cell death in the immediate posttransplant period. Ex vivo analysis demonstrated that sr39tk-expressing islets transplanted into the axillary cavity had normal morphology, were healthy and positive for insulin, glucagon, and somatostatin, and had no evidence of inflammation [70].

6. Combination of Imaging and Therapeutic Interventions

Therapeutic interventions for the enhancement of islet grafts performance can include immunomodulatory treatments of

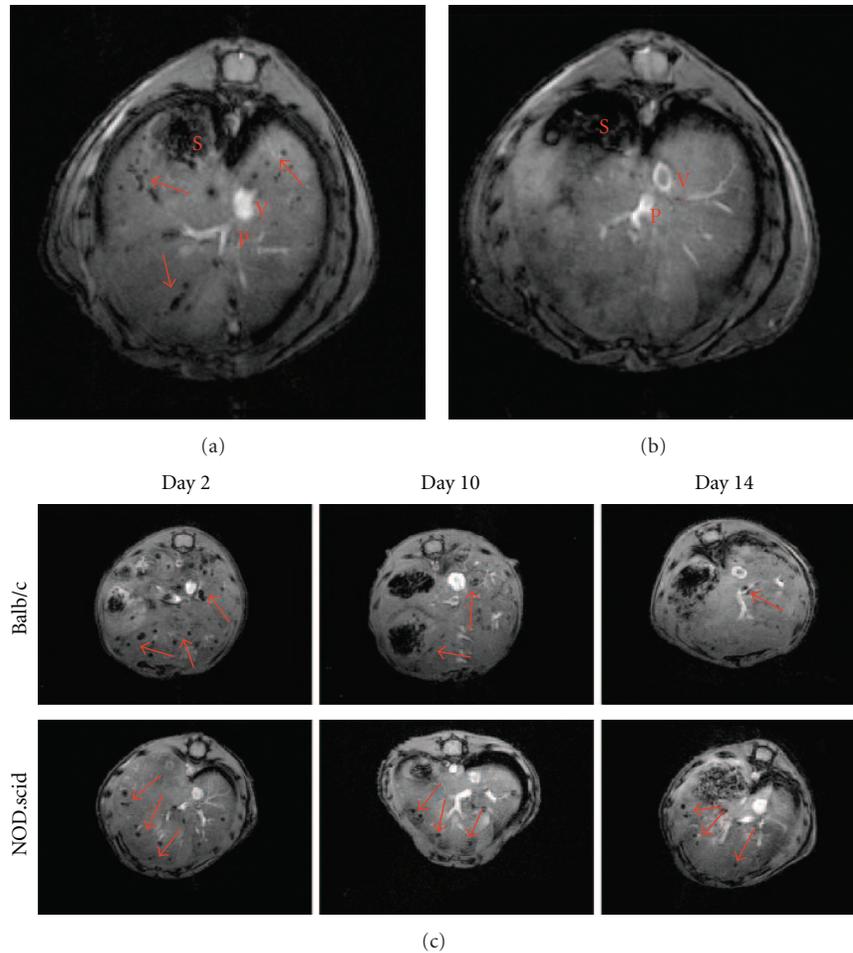


FIGURE 2: In vivo imaging of intrahepatically transplanted human islets. (a) Representative images of NOD.scid mice with transplanted islets. On in vivo images, Feridex-labeled islets appeared as signal voids scattered throughout the liver. (b) Nonlabeled islets were not detectable using the same imaging parameters. (c) In vivo time course imaging of immune rejection in immunocompetent (upper row) and immunocompromised (lower row) animals. Representative images are shown from days 2, 10, and 14 after transplantation. Note that the signal voids (arrows) representing labeled islets/islet clusters tend to disappear faster in Balb/c mice, reproduced with permission from American Diabetes Association [78].

the graft or of the host to minimize allorecognition or autoimmunity, methods to expedite graft revascularization, and approaches aimed at optimizing islet function or survival. The potential of noninvasive imaging to evaluate these treatments is an important part of their success. In addition, novel technologies may provide tools combining imaging and delivery of experimental therapies that aim to extend the lifespan and functionality of islet grafts.

6.1. Bioluminescence Imaging. In one of the earliest reports using BLI to evaluate a novel therapeutic strategy for transplanted islet immunoprotection, canine pancreatic islets were encapsulated into biocompatible alginate beads and transplanted into the dorsal-cervical fat pad of a nuclear factor-kappa beta (NF- κ B) luciferase transgenic mouse model [89]. Mice were imaged by BLI for up to 45 days after transplantation to evaluate the long-term inflammatory response provoked by the transplantation. The results

suggested that the trauma of surgery was a more significant inflammatory trigger than host immune responses to the capsules and that capsule size, rather than composition, correlated with an increase in inflammatory activity in this model. This study is valuable because it establishes the feasibility of a method that can be used to assess the extent to which various immunomodulatory strategies provoke an inflammatory response in the context of islet transplantation.

A more recent investigation attempted to determine whether early detection of rejection by BLI could aid in the timing of antilymphocyte serum (ALS) treatment for prolonging islet grafts survival [90]. Transgenic islets expressing the firefly luciferase were transplanted under the kidney capsule of streptozotocin-induced diabetic allogeneic immunocompetent mice. The animals received anti-lymphocyte therapy whose effect on islet survival was monitored by BLI. Imaging was proven useful in designing an optimal timing of therapy administration resulting in a close to 60% reduction

in grafts loss from rejection. Interestingly, the same success could not be achieved using blood glucose levels as a guide for the timing of therapy.

6.2. Nuclear Imaging. Nuclear imaging has been used to determine the feasibility of PET reporter gene (PRG) and PET reporter probe (PRP) technology for tracking islet grafts survival and quantifying the expression of potential therapeutic genes [71]. The authors generated a dual gene—expressing recombinant adenovirus rAD-vIL10-ITK, which coexpresses viral interleukin-10 (vIL-10) and HSV1-sr39tk. vIL-10 was chosen because it protects transplanted islets from immunological attack by regulating autoimmune activity. Islets from nondiabetic NOD mice were infected with rAD-vIL10-ITK or rAD-ITK, transplanted under the right kidney capsule of diabetic NOD mice, injected with [18F] FHBG, and scanned by microPET. PET signals in mice transplanted with islets infected with rAD-ITK (rAD-ITK mice) were decreased at 3 d and had almost reached basal values at 14 d after transplantation. Imaging revealed a relative extension of islet grafts survival in the presence of IL-10 treatment [71], illustrating the potential utility of anti-inflammatory therapy in islet transplantation.

6.3. Magnetic Resonance Imaging. Magnetic resonance imaging also has been explored for the pre-clinical assessment of therapeutic interventions in the context of islet transplantation. The first study was done in a large animal model (swine) and described the application of immunoprotective magnetocapsules containing ferumoxides, as carriers of pancreatic islets [81]. Islet-containing magnetocapsules were infused and engrafted into the liver. MRI could monitor this process in real time because of the incorporation of ferumoxides into the capsules.

A similar multifunctional rationale aimed at exploring the possibility for concurrently imparting imaging and therapeutic capabilities to transplanted pancreatic islets has also been behind some of our most recent work [92]. Taking advantage of the propensity of pancreatic islets to avidly take up dextran-coated superparamagnetic iron oxide nanoparticles, we designed a probe that consisted of a dextran-coated iron oxide core, conjugated to small interfering RNA (siRNA). Considering the potential of siRNA as a novel class of small molecule drugs capable of selectively silencing the expression of essentially any gene of choice with single nucleotide specificity, we speculated that we could explore the mechanism of RNA interference in the context of islet transplantation. As proof of concept, we showed that siRNA tagged to magnetic nanoparticles could accumulate in pancreatic islets in quantities sufficient for detection by MRI *in vitro* and for silencing target genes (green fluorescent protein [GFP] was used as a model gene) [92].

A more recent study by our group used a dual-purpose therapy/imaging nanoparticle probe to target the apoptotic-related gene caspase-3. We demonstrated that our “two-in-one” MN-siCaspase-3 imaging probe could silence the apoptotic-related gene, providing significant protection to

the grafts from early loss after transplantation, and at the same time served as an MRI label to assess the *in vivo* post-transplant fate of the grafts noninvasively (Figure 3) [24, 91]. The results of our study are in line with those of another recently published study showing that the use of fluorinated alginate microcapsules increased the insulin secretion rate of human islets and at the same time allowed detection by MRI and CT imaging [94].

In the context of islet transplantation, these studies are valuable because they lay the groundwork for future applications, in which genes implicated in islet grafts loss (immunological or nonimmunological) can be similarly targeted in order to improve graft outcome. Furthermore, the inherent imaging capabilities of the approach permit the noninvasive tracking of therapeutic moiety conjugated to the contrast agent and its relationship to graft fate.

7. Imaging of Metabolic Effects on Islet Grafts

One factor normally present during clinical transplantation and influencing transplanted islet fate is glucotoxicity caused by chronic hyperglycemia. To assess its input to the graft outcome, our group labeled human pancreatic islets with iron oxides and transplanted them into hyperglycemic and normoglycemic animals [95]. Noninvasive MRI monitored the fate of the grafts in the two groups. We found that in diabetic animals there was a significantly higher rate of islet death than in the normoglycemic counterparts. The half-life of an islet in the diabetic group was 4.8 ± 1.1 days compared with 12.6 ± 2.9 days for control nondiabetic mice ($P < 0.05$). This is the first *in vivo* study that confirms previous *in vitro* reports demonstrating that severe hyperglycemia impairs graft function and that successful islet transplantation depends on the degree of hyperglycemia in the recipient [96–103].

8. Imaging of Stem-Cell-Derived Insulin-Producing Cells

Pluripotent stem cells have the potential to differentiate into specialized cells of all three primary germ layers. Embryonic stem (ES) cells and the newly developed induced pluripotent stem (iPS) cells are an ideal source for generating insulin-producing cells (IPCs) that could be used to treat diabetes.

Most recently, BLI was used for monitoring ES cell survival and differentiation into insulin-producing cells in a diabetic animal model. This group generated a double transgenic mouse ES cell line ectopically expressing Pdx1-aequorea coeruleus green fluorescent protein (AcGFP) fusion protein, and rat insulin promoter- (RIP-) driven luciferase reporter [93, 104]. Real-time noninvasive BLI was used to monitor cell fate and function after transplantation. They speculated that, *in vivo*, pancreatic endoderm-like cells (PELCs) migrate into the streptozotocin-damaged pancreas and differentiate into IPCs. The *in vivo* differentiation of double transgenic ES cells transplanted under the renal capsule or systemically infused cells could be imaged by BLI as early as day 3 and until day 35 after-transplantation [93].

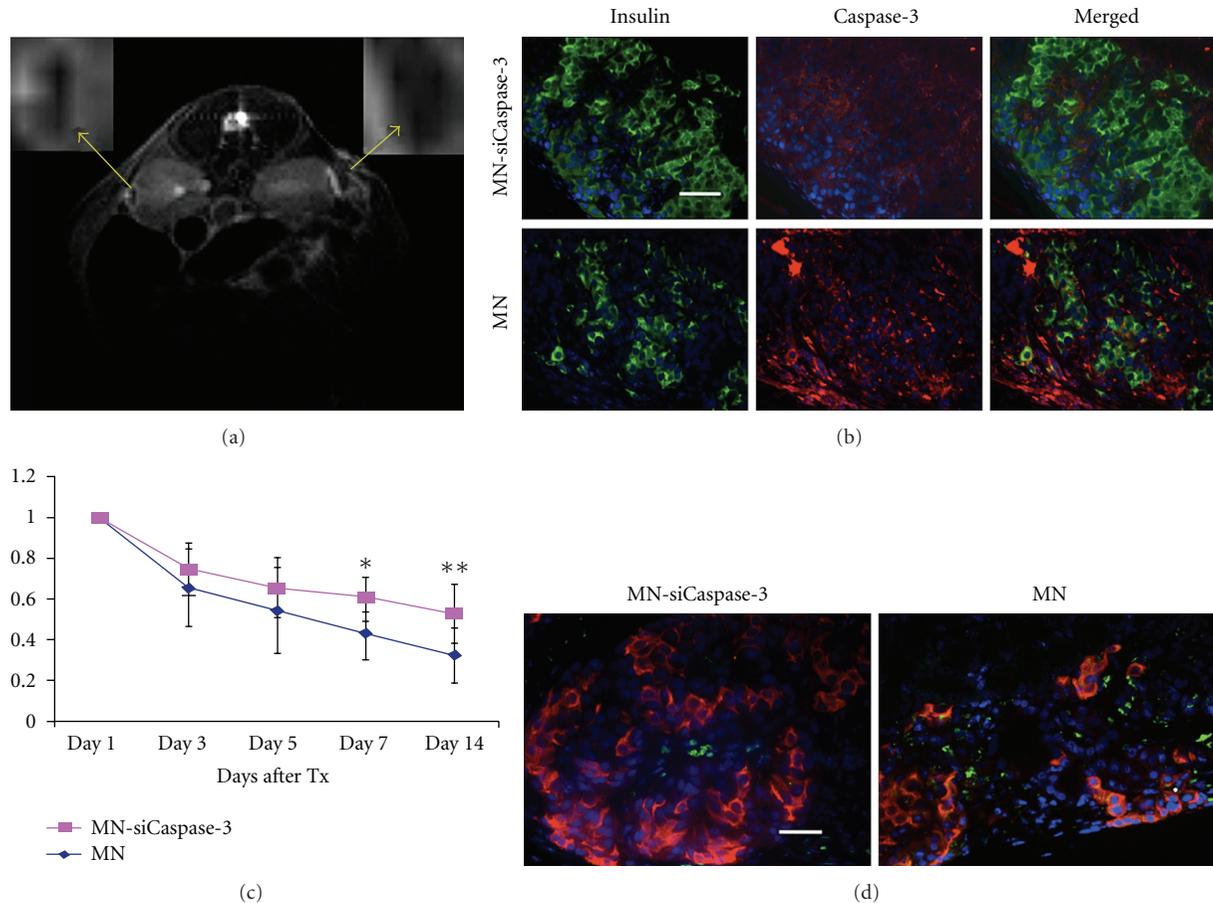


FIGURE 3: (a) Representative in vivo MRI of islet transplantation showing MN-siCaspase-3-treated islets implanted under the left kidney (inset) and parental MN-treated islets implanted under the right kidney. The dark area outlined under both kidney capsules represents the labeled grafts (day 3 shown). (b) Semiquantitative assessment of the relative changes in graft volumes revealed protective effect in MN-siCaspase-3-labeled grafts (*day 7, $P < 0.05$; **day 14, $P < 0.05$). (c) Fluorescence microscopy revealed higher expression of insulin and lower expression of caspase-3 in MN-siCaspase-3-treated grafts compared with MN-labeled islet grafts on the 14th day after-transplantation (Tx) (green, insulin; red, cleaved caspase-3; blue, DAPI nuclear stain) (magnification bar = 50 μm). (d): TUNEL assay on insulin-stained sections confirmed lower apoptotic rate and higher insulin expression in islets treated with MN-siCaspase-3 compared with islets treated with MN on the 14th day post-Tx (red, insulin; green, TUNEL; blue, DAPI nuclear stain) (magnification bar = 50 μm), reproduced with permission from American Diabetes Association [91].

9. Conclusions

Pancreatic islets, when transplanted, should be able to restore glucose homeostasis in the patient with diabetes. Nevertheless, the processes behind islet isolation, delivery, engraftment, and proper functioning in a new metabolic and immunological environment are complex and still poorly understood. Here, we have briefly discussed the important questions related to islet transplantation that could be tackled through in vivo molecular imaging. They bridge a range of topics, including graft longevity in the context of immune rejection and hyperglycemia, effects of autoimmunity and strategies to alleviate immune-mediated deterioration of graft viability and function, and the exploration of novel transplantation sites.

Still, despite the significant value of these studies, they reflect only a small proportion of the issues that face the field of islet transplantation. Questions that remain unresolved or

need to be studied in more detail span from the mechanics of islet engraftment and vascularization to the molecular aspects allowing islets to adapt to a new immunological, physiologic, and metabolic niche distinct from the pancreas, the importance of factors, such as islet innervation, ambient metabolite, ion, or signaling molecule concentrations for proper islet function, and so forth. Furthermore, having in mind the limited supply of donor tissue, another crucial subject to investigate would involve the potential of transplanting nonislet tissue, such as stem cells or cell types that can be induced to differentiate or transdifferentiate into functional islet tissue.

Since molecular imaging has the advantage of delivering quantitative temporal information in intact, authentic physiologic environments and on a systems' level, its potential for comprehensively addressing the issues of immediate interest to islet transplantation is apparent. Development of

probes targeting biomarkers linked to beta-cell function and combining therapeutic and diagnostic (theragnostics) tools for improving islet transplantation should be encouraged. With the first feasibility steps having been already made, the near future should see rapid progress.

Acknowledgments

This paper describes the work supported in part by NIH Grants 5RO1DK072137, 5RO1DK078615, and 1RO1DK080784 to A. Moore.

References

- [1] D. P. Group, "Incidence and trends of childhood type 1 diabetes worldwide 1990–1999," *Diabetic Medicine*, vol. 23, no. 8, pp. 857–866, 2006.
- [2] J. Azzi, A. S. Geara, S. El-Sayegh, and R. Abdi, "Immunological aspects of pancreatic islet cell transplantation," *Expert Review of Clinical Immunology*, vol. 6, no. 1, pp. 111–124, 2010.
- [3] W. D. Kelly, R. C. Lillehei, F. K. Merkel, Y. Idezuki, and F. G. Goetz, "Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy," *Surgery*, vol. 61, no. 6, pp. 827–837, 1967.
- [4] P. Fioretto, M. W. Steffes, D. E. R. Sutherland, F. C. Goetz, and M. Mauer, "Reversal of lesions of diabetic nephropathy after pancreas transplantation," *The New England Journal of Medicine*, vol. 339, no. 2, pp. 69–75, 1998.
- [5] R. Downing, "Historical review of pancreatic islet transplantation," *World Journal of Surgery*, vol. 8, no. 2, pp. 137–142, 1984.
- [6] P. E. Lacy and M. Kostianovsky, "Method for the isolation of intact islets of Langerhans from the rat pancreas," *Diabetes*, vol. 16, no. 1, pp. 35–39, 1967.
- [7] P. J. Hauptman and K. J. O'Connor, "Procurement and allocation of solid organs for transplantation," *The New England Journal of Medicine*, vol. 336, no. 6, pp. 422–431, 1997.
- [8] P. G. Stock and J. A. Bluestone, "Beta-cell replacement for type I diabetes," *Annual Review of Medicine*, vol. 55, pp. 133–156, 2004.
- [9] R. G. Bretzel, D. Brandhorst, H. Brandhorst et al., "Improved survival of intraportal pancreatic islet cell allografts in patients with type-1 diabetes mellitus by refined peritransplant management," *Journal of Molecular Medicine*, vol. 77, no. 1, pp. 140–143, 1999.
- [10] A. M. J. Shapiro, J. R. T. Lakey, E. A. Ryan et al., "Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen," *The New England Journal of Medicine*, vol. 343, no. 4, pp. 230–238, 2000.
- [11] R. Alejandro, F. B. Barton, B. J. Hering, and S. Wease, "2008 update from the collaborative islet transplant registry," *Transplantation*, vol. 86, no. 12, pp. 1783–1788, 2008.
- [12] A. M. J. Shapiro, C. Ricordi, B. J. Hering et al., "International trial of the Edmonton protocol for islet transplantation," *The New England Journal of Medicine*, vol. 355, no. 13, pp. 1318–1330, 2006.
- [13] E. A. Ryan, B. W. Paty, P. A. Senior et al., "Five-year follow-up after clinical islet transplantation," *Diabetes*, vol. 54, no. 7, pp. 2060–2069, 2005.
- [14] N. R. Barshes, S. Wyllie, and J. A. Goss, "Inflammation-mediated dysfunction and apoptosis in pancreatic islet transplantation: implications for intrahepatic grafts," *Journal of Leukocyte Biology*, vol. 77, no. 5, pp. 587–597, 2005.
- [15] S. Lacotte, T. Berney, A. J. Shapiro, and C. Toso, "Immune monitoring of pancreatic islet graft: towards a better understanding, detection and treatment of harmful events," *Expert Opinion on Biological Therapy*, vol. 11, no. 1, pp. 55–66, 2011.
- [16] M. D. Stegall, K. J. Lafferty, I. Sam, and R. G. Gill, "Evidence of recurrent autoimmunity in human allogeneic islet transplantation," *Transplantation*, vol. 61, no. 8, pp. 1272–1274, 1996.
- [17] C. Jaeger, B. J. Hering, E. Hatziagelaki, K. Federlin, and R. G. Bretzel, "Glutamic acid decarboxylase antibodies are more frequent than islet cell antibodies in islet transplanted IDDM patients and persist or occur despite immunosuppression," *Journal of Molecular Medicine*, vol. 77, no. 1, pp. 45–48, 1999.
- [18] N. Niclauss, D. Bosco, P. Morel, L. Giovannoni, T. Berney, and G. Parnaud, "Rapamycin impairs proliferation of transplanted islet β cells," *Transplantation*, vol. 91, no. 7, pp. 714–722, 2011.
- [19] E. A. Ryan, J. R. T. Lakey, R. V. Rajotte et al., "Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol," *Diabetes*, vol. 50, no. 4, pp. 710–719, 2001.
- [20] A. Pileggi, C. Ricordi, M. Alessiani, and L. Inverardi, "Factors influencing islet of Langerhans graft function and monitoring," *Clinica Chimica Acta*, vol. 310, no. 1, pp. 3–16, 2001.
- [21] L. Castano and G. S. Eisenbarth, "Type-I diabetes: a chronic autoimmune disease of human, mouse, and rat," *Annual Review of Immunology*, vol. 8, pp. 647–679, 1990.
- [22] M. A. Atkinson and N. K. Maclaren, "Mechanisms of disease: the pathogenesis of insulin-dependent diabetes mellitus," *The New England Journal of Medicine*, vol. 331, no. 21, pp. 1428–1436, 1994.
- [23] T. F. Massoud and S. S. Gambhir, "Molecular imaging in living subjects: seeing fundamental biological processes in a new light," *Genes and Development*, vol. 17, no. 5, pp. 545–580, 2003.
- [24] M. Gotthardt, "A therapeutic insight in β -cell imaging?" *Diabetes*, vol. 60, no. 2, pp. 381–382, 2011.
- [25] A. Moore, Z. Medarova, P. Vallabhajosyula et al., "In vivo imaging of autologous islet grafts in the liver and under the kidney capsule in non-human primates," *Transplantation*, vol. 87, no. 11, pp. 1659–1666, 2009.
- [26] M. Hara, X. Wang, T. Kawamura et al., "Transgenic mice with green fluorescent protein-labeled pancreatic β -cells," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 284, no. 1, pp. E177–E183, 2003.
- [27] Z. Medarova, S. Bonner-Weir, M. Lipes, and A. Moore, "Imaging β -cell death with a near-infrared probe," *Diabetes*, vol. 54, no. 6, pp. 1780–1788, 2005.
- [28] C. Ran, P. Pantazopoulos, Z. Medarova, and A. Moore, "Synthesis and testing of beta-cell-specific streptozotocin-derived near-infrared imaging probes," *Angewandte Chemie—International Edition*, vol. 46, no. 47, pp. 8998–9001, 2007.
- [29] T. Reiner, R. H. Kohler, C. W. Liew et al., "Near-infrared fluorescent probe for imaging of pancreatic β cells," *Bioconjugate Chemistry*, vol. 21, no. 7, pp. 1362–1368, 2010.
- [30] J. Virostko, A. Radhika, G. Poffenberger et al., "Bioluminescence imaging in mouse models quantifies β cell mass in the pancreas and after islet transplantation," *Molecular Imaging and Biology*, vol. 12, no. 1, pp. 42–53, 2010.

- [31] A. Moore, S. Bonner-Weir, and R. Weissleder, "Noninvasive in vivo measurement of β -cell mass in mouse model of diabetes," *Diabetes*, vol. 50, no. 10, pp. 2231–2236, 2001.
- [32] L. Ladrière, F. Malaisse-Lagae, R. Alejandro, and W. J. Malaisse, "Pancreatic fate of a ^{125}I -labelled mouse monoclonal antibody directed against pancreatic B-cell surface ganglioside(s) in control and diabetic rats," *Cell Biochemistry and Function*, vol. 19, no. 2, pp. 107–115, 2001.
- [33] K. N. Samli, M. J. McGuire, C. B. Newgard, S. A. Johnston, and K. C. Brown, "Peptide-mediated targeting of the islets of Langerhans," *Diabetes*, vol. 54, no. 7, pp. 2103–2108, 2005.
- [34] C. S. Hampe, A. R. Wallen, M. Schlosser, M. Ziegler, and I. R. Sweet, "Quantitative evaluation of a monoclonal antibody and its fragment as potential markers for pancreatic beta cell mass," *Experimental and Clinical Endocrinology and Diabetes*, vol. 113, no. 7, pp. 381–387, 2005.
- [35] S. Ueberberg, J. J. Meier, C. Waengler et al., "Generation of novel single-chain antibodies by phage-display technology to direct imaging agents highly selective to pancreatic β - or α -cells in vivo," *Diabetes*, vol. 58, no. 10, pp. 2324–2334, 2009.
- [36] M. Anlauf, R. Eissele, M. K.-H. Schäfer et al., "Expression of the two isoforms of the vesicular monoamine transporter (VMAT1 and VMAT2) in the endocrine pancreas and pancreatic endocrine tumors," *Journal of Histochemistry and Cytochemistry*, vol. 51, no. 8, pp. 1027–1040, 2003.
- [37] D. J. Pettibone, J. A. Totaro, and A. B. Pflueger, "Tetra-benzazine-induced depletion of brain monoamines: characterization and interaction with selected antidepressants," *European Journal of Pharmacology*, vol. 102, no. 3-4, pp. 425–430, 1984.
- [38] M. Freeby, R. Goland, M. Ichise, A. Maffei, R. Leibel, and P. E. Harris, "VMAT2 quantitation by PET as a biomarker for β -cell mass in health and disease," *Diabetes, Obesity and Metabolism*, vol. 10, supplement 4, pp. 98–108, 2008.
- [39] L. Zhu, J. Liu, and H. F. Kung, "Synthesis and evaluation of 2-amino-dihydro-tetrazine derivatives as probes for imaging vesicular monoamine transporter-2," *Bioorganic and Medicinal Chemistry Letters*, vol. 19, no. 17, pp. 5026–5028, 2009.
- [40] M. P. Kung, C. Hou, B. P. Lieberman et al., "In vivo imaging of β -cell mass in rats using 18F-FP-(+)-DTBZ: a potential PET ligand for studying diabetes mellitus," *Journal of Nuclear Medicine*, vol. 49, no. 7, pp. 1171–1176, 2008.
- [41] J. Eng, W. A. Kleinman, L. Singh, G. Singh, and J. P. Raufman, "Isolation and characterization of exendin-4, an exendin-3 analogue, from *Heloderma suspectum* venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas," *Journal of Biological Chemistry*, vol. 267, no. 11, pp. 7402–7405, 1992.
- [42] M. Gotthardt, M. Fischer, I. Naeher et al., "Use of the incretin hormone glucagon-like peptide-1 (GLP-1) for the detection of insulinomas: initial experimental results," *European Journal of Nuclear Medicine and Molecular Imaging*, vol. 29, no. 5, pp. 597–606, 2002.
- [43] M. Brom, K. Andraóć, W. J. G. Oyen, O. C. Boerman, and M. Gotthardt, "Development of radiotracers for the determination of the beta-cell mass in vivo," *Current Pharmaceutical Design*, vol. 16, no. 14, pp. 1561–1567, 2010.
- [44] F. Pattou, J. Kerr-Conte, and D. Wild, "GLP-1-receptor scanning for imaging of human beta cells transplanted in muscle," *The New England Journal of Medicine*, vol. 363, no. 13, pp. 1289–1290, 2010.
- [45] L. Ladrière, F. Malaisse-Lagae, and W. J. Malaisse, "Uptake of tritiated glibenclamide by endocrine and exocrine pancreas," *Endocrine*, vol. 13, no. 1, pp. 133–136, 2000.
- [46] A. Schmitz, C. Y. Shiue, Q. Feng et al., "Synthesis and evaluation of fluorine-18 labeled glyburide analogs as β -cell imaging agents," *Nuclear Medicine and Biology*, vol. 31, no. 4, pp. 483–491, 2004.
- [47] S. Schneider, P. J. Feilen, M. Schreckenberger et al., "In vitro and in vivo evaluation of novel glibenclamide derivatives as imaging agents for the non-invasive assessment of the pancreatic islet cell mass in animals and humans," *Experimental and Clinical Endocrinology and Diabetes*, vol. 113, no. 7, pp. 388–395, 2005.
- [48] I. R. Sweet, D. L. Cook, Å. Lernmark et al., "Systematic screening of potential β -cell imaging agents," *Biochemical and Biophysical Research Communications*, vol. 314, no. 4, pp. 976–983, 2004.
- [49] L. Ladrière, V. Leclercq-Meyer, and W. J. Malaisse, "Assessment of islet β -cell mass in isolated rat pancreases perfused with D-[3H]mannoheptulose," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 281, no. 2, pp. E298–E303, 2001.
- [50] W. J. Malaisse and L. Ladrière, "Assessment of B-cell mass in isolated islets exposed to D-[3H]mannoheptulose," *International Journal of Molecular Medicine*, vol. 7, no. 4, pp. 405–406, 2001.
- [51] W. J. Malaisse, M. Doherty, M. M. Kadiata, L. Ladrière, and F. Malaisse-Lagae, "Pancreatic fate of D-[3H] mannoheptulose," *Cell Biochemistry and Function*, vol. 19, no. 3, pp. 171–179, 2001.
- [52] Q. Zheng, H. Dai, M. Merritt et al., "Magnetic relaxation agents for cell labeling and imaging applications," in *Imaging the Pancreatic Beta Cell*, Bethesda, Md, USA, 2003.
- [53] A. Sambanis, C. Stabler, S. Tang et al., "Development and non-invasive monitoring of a pancreatic tissue substitute," in *Imaging the Pancreatic Beta Cell*, Bethesda, Md, USA, 2003.
- [54] I. Constantinidis, J. R. R. Long, C. Weber, S. Safley, and A. Sambanis, "Non-invasive monitoring of a bioartificial pancreas in vitro and in vivo," *Annals of the New York Academy of Sciences*, vol. 944, pp. 83–96, 2001.
- [55] D. Lu, H. Mulder, P. Zhao et al., " ^{13}C NMR isotopomer analysis reveals a connection between pyruvate cycling and glucose-stimulated insulin secretion (GSIS)," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 5, pp. 2708–2713, 2002.
- [56] B. Gimi, L. Leoni, S. Eroglu et al., "Imaging of pancreatic beta-cell function by Mn $^{2+}$ -enhanced MRI," in *Imaging the Pancreatic Beta Cell*, Bethesda, Md, USA, 2003.
- [57] B. Gimi, L. Leoni, J. Oberholzer et al., "Functional MR microimaging of pancreatic β -cell activation," *Cell Transplantation*, vol. 15, no. 2, pp. 195–203, 2006.
- [58] P. F. Antkowiak, S. A. Tersey, J. D. Carter et al., "Noninvasive assessment of pancreatic β -cell function in vivo with manganese-enhanced magnetic resonance imaging," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 296, no. 3, pp. E573–E578, 2009.
- [59] J. Yong, J. Rasooly, H. Dang et al., "Multimodality imaging of β -cells in mouse models of type 1 and 2 diabetes," *Diabetes*, vol. 60, no. 5, pp. 1383–1392, 2011.
- [60] D. Kaufman, M. Baker, J. Nelson et al., "In vivo, real-time, non-invasive bioluminescent imaging of transplanted islets in a functional murine model," in *Imaging the Pancreatic Beta Cell*, Bethesda, Md, USA, 2003.

- [61] A. Powers, M. Fowler, J. Virostko et al., "Using bioluminescence to non-invasively image and assess transplanted islet mass," in *Imaging the Pancreatic Beta Cell*, Bethesda, Md, USA, 2003.
- [62] Y. Lu, H. Dang, B. Middleton et al., "Bioluminescent monitoring of islet graft survival after transplantation," *Molecular Therapy*, vol. 9, no. 3, pp. 428–435, 2004.
- [63] M. Fowler, J. Virostko, Z. Chen et al., "Assessment of pancreatic islet mass after islet transplantation using in vivo bioluminescence imaging," *Transplantation*, vol. 79, no. 7, pp. 768–776, 2005.
- [64] Y. A. Cao, M. H. Bachmann, A. Beilhack et al., "Molecular imaging using labeled donor tissues reveals patterns of engraftment, rejection, and survival in transplantation," *Transplantation*, vol. 80, no. 1, pp. 134–139, 2005.
- [65] X. Chen, X. Zhang, C. S. Larson, M. S. Baker, and D. B. Kaufman, "In vivo bioluminescence imaging of transplanted islets and early detection of graft rejection," *Transplantation*, vol. 81, no. 10, pp. 1421–1427, 2006.
- [66] S. Bertera, X. Geng, Z. Tawadrous et al., "Body window-enabled in vivo multicolor imaging of transplanted mouse islets expressing an insulin-timer fusion protein," *BioTechniques*, vol. 35, no. 4, pp. 718–722, 2003.
- [67] N. V. Evgenov, Z. Medarova, G. Dai, S. Bonner-Weir, and A. Moore, "In vivo imaging of islet transplantation," *Nature Medicine*, vol. 12, no. 1, pp. 144–148, 2006.
- [68] O. Eriksson, T. Eich, A. Sundin et al., "Positron emission tomography in clinical islet transplantation," *American Journal of Transplantation*, vol. 9, no. 12, pp. 2816–2824, 2009.
- [69] Y. Lu, H. Dang, B. Middleton et al., "Repetitive microPET imaging of implanted human islets in mice," in *Imaging the Pancreatic Beta Cell*, Bethesda, Md, USA, 2003.
- [70] Y. Lu, H. Dang, B. Middleton et al., "Long-term monitoring of transplanted islets using positron emission tomography," *Molecular Therapy*, vol. 14, no. 6, pp. 851–856, 2006.
- [71] S. J. Kim, D. J. Doudet, A. R. Studenov et al., "Quantitative micro positron emission tomography (PET) imaging for the in vivo determination of pancreatic islet graft survival," *Nature Medicine*, vol. 12, no. 12, pp. 1423–1428, 2006.
- [72] C. Toso, H. Zaidi, P. Morel et al., "Positron-emission tomography imaging of early events after transplantation of islets of Langerhans," *Transplantation*, vol. 79, no. 3, pp. 353–355, 2005.
- [73] T. Eich, O. Eriksson, A. Sundin et al., "Positron emission tomography: a real-time tool to quantify early islet engraftment in a preclinical large animal model," *Transplantation*, vol. 84, no. 7, pp. 893–898, 2007.
- [74] T. Eich, O. Eriksson, and T. Lundgren, "Visualization of early engraftment in clinical islet transplantation by positron-emission tomography," *The New England Journal of Medicine*, vol. 356, no. 26, pp. 2754–2755, 2007.
- [75] T. Shen, R. Weissleder, M. Papisov, A. Bogdanov, and T. J. Brady, "Monocrystalline iron oxide nanocompounds (MION): physicochemical properties," *Magnetic Resonance in Medicine*, vol. 29, no. 5, pp. 599–604, 1993.
- [76] D. Jiráková, J. Kríz, V. Herynek et al., "MRI of transplanted pancreatic islets," *Magnetic Resonance in Medicine*, vol. 52, no. 6, pp. 1228–1233, 2004.
- [77] L. Biancone, S. G. Crich, V. Cantaluppi et al., "Magnetic resonance imaging of gadolinium-labeled pancreatic islets for experimental transplantation," *NMR in Biomedicine*, vol. 20, no. 1, pp. 40–48, 2007.
- [78] N. V. Evgenov, Z. Medarova, J. Pratt et al., "In vivo imaging of immune rejection in transplanted pancreatic islets," *Diabetes*, vol. 55, no. 9, pp. 2419–2428, 2006.
- [79] T. Koblas, P. Girman, Z. Berkova et al., "Magnetic resonance imaging of intrahepatically transplanted islets using paramagnetic beads," *Transplantation Proceedings*, vol. 37, no. 8, pp. 3493–3495, 2005.
- [80] H. T. Joo, P. Foster, A. Rosales et al., "Imaging islets labeled with magnetic nanoparticles at 1.5 Tesla," *Diabetes*, vol. 55, no. 11, pp. 2931–2938, 2006.
- [81] B. P. Barnett, A. Arepally, P. V. Karmarkar et al., "Magnetic resonance-guided, real-time targeted delivery and imaging of magnetocapsules immunoprotecting pancreatic islet cells," *Nature Medicine*, vol. 13, no. 8, pp. 986–991, 2007.
- [82] C. Toso, J. P. Vallee, P. Morel et al., "Clinical magnetic resonance imaging of pancreatic islet grafts after iron nanoparticle labeling," *American Journal of Transplantation*, vol. 8, no. 3, pp. 701–706, 2008.
- [83] Z. Fan, J. A. Spencer, Y. Lu et al., "In vivo tracking of 'color-coded' effector, natural and induced regulatory T cells in the allograft response," *Nature Medicine*, vol. 16, no. 6, pp. 718–722, 2010.
- [84] C. Ricordi and T. B. Strom, "Clinical islet transplantation: advances and immunological challenges," *Nature Reviews Immunology*, vol. 4, no. 4, pp. 259–268, 2004.
- [85] X. Chen, X. Zhang, C. Larson, F. Chen, H. Kissler, and D. B. Kaufman, "The epididymal fat pad as a transplant site for minimal islet mass," *Transplantation*, vol. 84, no. 1, pp. 122–125, 2007.
- [86] S. Speier, D. Nyqvist, O. Cabrera et al., "Noninvasive in vivo imaging of pancreatic islet cell biology," *Nature Medicine*, vol. 14, no. 5, pp. 574–578, 2008.
- [87] S. Speier, D. Nyqvist, M. Köhler, A. Caicedo, I. B. Leibiger, and P. O. Berggren, "Noninvasive high-resolution in vivo imaging of cell biology in the anterior chamber of the mouse eye," *Nature Protocols*, vol. 3, no. 8, pp. 1278–1286, 2008.
- [88] M. H. Abdulreda, G. Faleo, R. D. Molano et al., "High-resolution, noninvasive longitudinal live imaging of immune responses," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 31, pp. 12863–12868, 2011.
- [89] D. J. Roth, E. D. Jansen, A. C. Powers, and T. G. Wang, "A novel method of monitoring response to islet transplantation: bioluminescent imaging of an NF- κ B transgenic mouse model," *Transplantation*, vol. 81, no. 8, pp. 1185–1190, 2006.
- [90] X. Chen, X. Zhang, C. Larson, G. Xia, and D. B. Kaufman, "Prolonging islet allograft survival using in vivo bioluminescence imaging to guide timing of antilymphocyte serum treatment of rejection," *Transplantation*, vol. 85, no. 9, pp. 1246–1252, 2008.
- [91] P. Wang, M. V. Yigit, Z. Medarova et al., "Combined small interfering RNA therapy and in vivo magnetic resonance imaging in islet transplantation," *Diabetes*, vol. 60, no. 2, pp. 565–571, 2011.
- [92] Z. Medarova, M. Kumar, S. W. Ng et al., "Multifunctional magnetic nanocarriers for image-tagged siRNA delivery to intact pancreatic islets," *Transplantation*, vol. 86, no. 9, pp. 1170–1177, 2008.
- [93] S. P. Raikwar and N. Zavazava, "Spontaneous in vivo differentiation of embryonic stem cell-derived pancreatic endoderm-like cells corrects hyperglycemia in diabetic mice," *Transplantation*, vol. 91, no. 1, pp. 11–20, 2011.
- [94] B. P. Barnett, J. Ruiz-Cabello, P. Hota et al., "Fluorocapsules for improved function, immunoprotection, and visualization

- of cellular therapeutics with MR, US, and CT imaging,” *Radiology*, vol. 258, no. 1, pp. 182–191, 2011.
- [95] N. V. Evgenov, J. Pratt, P. Pantazopoulos, and A. Moore, “Effects of glucose toxicity and islet purity on in vivo magnetic resonance imaging of transplanted pancreatic islets,” *Transplantation*, vol. 85, no. 8, pp. 1091–1098, 2008.
- [96] A. M. Davalli, L. Scaglia, D. H. Zangen, J. Hollister, S. Bonner-Weir, and G. C. Weir, “Vulnerability of islets in the immediate posttransplantation period: dynamic changes in structure and function,” *Diabetes*, vol. 45, no. 9, pp. 1161–1167, 1996.
- [97] M. Biarnés, M. Montolio, V. Nacher, M. Raurell, J. Soler, and E. Montanya, “ β -cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia,” *Diabetes*, vol. 51, no. 1, pp. 66–72, 2002.
- [98] E. Montana, S. Bonner-Weir, and G. C. Weir, “Beta cell mass and growth after syngeneic islet cell transplantation in normal and streptozocin diabetic C57BL/6 mice,” *Journal of Clinical Investigation*, vol. 91, no. 3, pp. 780–787, 1993.
- [99] L. Makhlouf, V. F. Duvivier-Kali, S. Bonner-Weir, H. Di-eperink, G. C. Weir, and M. H. Sayegh, “Importance of hyperglycemia on the primary function of allogeneic islet transplants,” *Transplantation*, vol. 76, no. 4, pp. 657–664, 2003.
- [100] V. Nacher, J. F. Merino, M. Raurell, J. Soler, and E. Montanya, “Normoglycemia restores β -cell replicative response to glucose in transplanted islets exposed to chronic hyperglycemia,” *Diabetes*, vol. 47, no. 2, pp. 192–196, 1998.
- [101] R. Melzi, M. Battaglia, E. Draghici, E. Bonifacio, and L. Piemonti, “Relevance of hyperglycemia on the timing of functional loss of allogeneic islet transplants: implication for mouse model,” *Transplantation*, vol. 83, no. 2, pp. 167–173, 2007.
- [102] D. L. Eizirik, L. Jansson, M. Flodström, C. Hellerström, and A. Andersson, “Mechanisms of defective glucose-induced insulin release in human pancreatic islets transplanted to diabetic nude mice,” *Journal of Clinical Endocrinology and Metabolism*, vol. 82, no. 8, pp. 2660–2663, 1997.
- [103] L. Jansson, D. L. Eizirik, D. G. Pipeleers, L. A. H. Borg, C. Hellerstrom, and A. Andersson, “Impairment of glucose-induced insulin secretion in human pancreatic islets transplanted to diabetic nude mice,” *Journal of Clinical Investigation*, vol. 96, no. 2, pp. 721–726, 1995.
- [104] S. P. Raikwar and N. Zavazava, “Real-time non-invasive imaging of ES cell-derived insulin producing cells,” *Methods in Molecular Biology*, vol. 590, pp. 317–334, 2009.

Review Article

B7-H4 Pathway in Islet Transplantation and β -Cell Replacement Therapies

Xiaojie Wang,¹ Jianqiang Hao,¹ Daniel L. Metzger,² Ziliang Ao,¹ Mark Meloche,¹ C. Bruce Verchere,³ Lieping Chen,⁴ Dawei Ou,¹ Alice Mui,¹ and Garth L. Warnock¹

¹ Department of Surgery, University of British Columbia, Vancouver, BC, Canada V5Z 4E3

² Department of Paediatrics, University of British Columbia, Vancouver, BC, Canada V6H 3V4

³ Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada V5Z 4H4

⁴ Department of Immunobiology, Yale University School of Medicine, New Haven, CT 06519, USA

Correspondence should be addressed to Garth L. Warnock, gwarnock@interchange.ubc.ca

Received 21 April 2011; Accepted 3 August 2011

Academic Editor: Andrew Posselt

Copyright © 2011 Xiaojie Wang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Type 1 diabetes (T1D) is a chronic autoimmune disease and characterized by absolute insulin deficiency. β -cell replacement by islet cell transplantation has been established as a feasible treatment option for T1D. The two main obstacles after islet transplantation are alloreactive T-cell-mediated graft rejection and recurrence of autoimmune diabetes mellitus in recipients. T cells play a central role in determining the outcome of both autoimmune responses and allograft survival. B7-H4, a newly identified B7 homolog, plays a key role in maintaining T-cell homeostasis by reducing T-cell proliferation and cytokine production. The relationship between B7-H4 and allograft survival/autoimmunity has been investigated recently in both islet transplantation and the nonobese diabetic (NOD) mouse models. B7-H4 protects allograft survival and generates donor-specific tolerance. It also prevents the development of autoimmune diabetes. More importantly, B7-H4 plays an indispensable role in alloimmunity in the absence of the classic CD28/CTLA-4 : B7 pathway, suggesting a synergistic/additive effect with other agents such as CTLA-4 on inhibition of unwanted immune responses.

1. Introduction

Type 1 diabetes (T1D) is fatal unless treated with insulin. Injection of insulin prevents the hyperglycemic complications of T1D, including ketoacidosis and coma. However, exogenous administration of excessive or inadequate amounts of insulin often results in hypo- and hyperglycemia, respectively [1–3]. Combination of intensive glycemic monitoring and best medical therapy provides better control of insulin level and reduces the microvascular and macrovascular complications of diabetes, but it also increases the risk of severe hypoglycemia [1–3]. In selected patients, islet transplantation is a reasonable therapeutic option. Restoration of β -cell mass by whole-pancreas or islet cell transplantation provides physiologically regulated insulin as well as other hormones, such as glucagon to avoid life-threatening unregulated glucose levels [4–6]. Whole-pancreas transplantation is a major surgical procedure and is usually performed in

conjunction with a kidney transplant, either simultaneous pancreas-kidney transplant (SPK) or pancreas-after-kidney transplant (PAK), for patients with end-stage renal disease. By contrast, islet cell transplantation is a less invasive and relatively simple procedure. Isolated islets are infused into the liver through the portal vein, guided by fluoroscopic cannulation. The one-year success rate is comparable for both types of transplantations. Significant progress has been made in islet transplantation, especially the establishment of the “Edmonton protocol” in 2000 [7]. This success of this protocol is based on provision of both sufficient islet-cell mass and a steroid-free immunosuppressive regimen. However, the subsequent followups conducted worldwide in several centers show a constant decline in graft function [8]. Despite this, the majority of recipients benefit from reduced overall insulin requirements; improved C-peptide secretion and HbA1C levels; decreased development of microvascular complications; and fewer complications related to episodes

respectively. Once differentiated, each lineage secretes a specific cytokine profile. For example, Th1 and Th2 subsets secrete interferon γ (IFN- γ) and IL-4/IL-10, respectively [14]. The Th1, Th2, Treg, and Th17 subsets cooperate and influence the outcome of transplanted grafts. The Th1 cytokine profile was previously thought to be associated with allograft damage and rejection, while the Th2 profile favors the acquisition of protection and tolerance [20]. However, this simplified Th1/Th2 paradigm may not be sufficient to explain redundant effects of cytokine networks on the outcome of transplantation *in vivo*.

The newly characterized regulatory T-cell subset, Tregs, plays an anti-inflammatory role and maintains tolerance to self-antigens. Tregs (CD4⁺ CD25⁺) suppress proliferation of CD4⁺ CD25⁻ T cells, CD8⁺ T cells, dendritic cells, B cells, macrophages, mast cells, osteoblasts, NK, and NKT cells in an antigen-non-specific manner [21]. Tregs play an important role in preventing transplant rejection and generating tolerance [22].

3. Tregs and Transplantation

Treg-mediated tolerance induction has been well established as a nondeletional strategy to modulate alloreactive T-cell responses. The role of Treg in maintaining self-tolerance was described by Sakaguchi's group, which showed that Tregs from the thymus protect the host from autoimmune diseases [23].

Tregs can be categorized into two main types (naturally occurring Tregs, nTregs, and inducible Tregs, iTregs) based on their origins, mechanisms, and modes of action [24]. They are both called regulatory T cells because they have similarities in terms of phenotype and function. First, both express Foxp3 and suppress the proliferation of effector cells. Secondly, Tregs will be absent from the thymus or the periphery, if proximal TCR signaling is disrupted, either through genetic manipulation or the use of calcineurin inhibitors. Thirdly, the development of both types of Tregs is dependent on cytokines such as IL-2. The two subsets of Tregs can be differentiated by their distinct suppressive mechanisms and other characteristics. nTregs develop in the thymus and constitute approximately 5–10% of peripheral CD4⁺ T cells. CD4⁺CD25⁺ Tregs suppress effector T-cell proliferation *in vitro* through a cell contact-dependent mechanism, and their function is cytokine-independent [24]. A role for nTregs in the development of transplantation tolerance was first indicated by their ability to suppress mouse GVHD following adoptive transfer [25].

The second population of Treg subsets (iTregs) is distinct from nTregs and arises during immune responses in the periphery. iTregs suppress immune responses through secretion of immunosuppressive cytokines. Th3 and Tr1 cells induce suppression through secretion of TGF- β and IL-10, respectively, [24]. Tr1 was first identified by Groux et al. [26]. Naïve T cells from ovalbumin (OVA) TCR-transgenic mice stimulated with OVA and IL-10 suppress antigen-specific activation *in vitro* and prevent the development of colitis *in vivo* [26]. Moreover, the supernatant from Tr1 strongly

suppresses alloantigen-specific T-cell proliferation [27]. Tr1 cells tend to migrate toward the site of inflammation. Tregs can be detected in the extralymphoid sites. It shows that CD4⁺CD25⁺ Tregs are overexpressed within tolerated allograft [28]. Th3 was originally described in oral tolerance in mice induced by myelin basic protein (MBP) [29]. Th3 suppressive cells may be converted from nonregulatory cells in the presence of TGF- β [30]. The existence of suppressive cells of nonthymus origin was confirmed by the demonstration of CD4⁺CD25⁺ conversion from CD25⁻ precursors in thymectomized mice, and that these non-thymus-derived Tregs can suppress skin allograft rejection [31].

4. Costimulation Blockade in Transplantation

T cells are the principal mediator of alloreactive reactions, and their full activation requires 2 signals. The first is provided by the interaction of antigen-specific TCRs and their cognate alloantigens presented on MHCs. The second signal is antigen nonspecific. It can be provided *in trans* by APCs and requires cell-cell contact [32]. Signal 1 alone results in no response. Signals 1 plus 2 lead to either activation or inhibition of the T-cell response, depending on which cosignal pathway dominates. In the presence of positive signals, such as CD28, T cells become activated upon stimulation with foreign antigen. On the contrary, activated T-cell proliferation is terminated with negative coinhibitory signals, such as CTLA-4 [33]. The requirement of two separate signals for full activation of the intracellular signalling cascade, IL-2 transcription, T-cell proliferation, and effector function suggests a critical role of cosignalling pathways in determining the fate of transplantation (Figure 2). Much attention has been focused on using agents which are either coinhibitory with negative-signal molecules, or antagonistic to positive-signal molecules, to control allograft rejection.

The discovery of the requirement of both recognition signal (signal 1) and verification signal (signal 2) for effective T-cell activation has elucidated potential targets for immunosuppression that are highly T-cell specific. In particular, the need for additional cosignalling to avoid anergy or apoptosis has generated the strategy for tolerance induction using costimulation blockade. In fact, T-cell clones cultured with antigen and MHC alone lead to unresponsiveness [34]; in other words, T cells will undergo anergy or apoptosis without signal 2. This observation suggests the attractive prospect that allografts could become tolerated if signal 2 is blocked. Although diverse strategies have been investigated to enhance graft survival in terms of improved efficacy and reduced toxicity, different degrees of side effects are unavoidable. Therefore, tolerance induction or withdrawal of long-term usage of immunosuppressants is preferred. Theoretically, it may be achieved by using costimulation blockade.

5. B7-H4 Coinhibitory Pathway

B7-H4 (B7x or B7S) was identified by three independent groups using expressed sequence tags (EST) with homology to the B7 family [35–37]. The genes for human and mouse

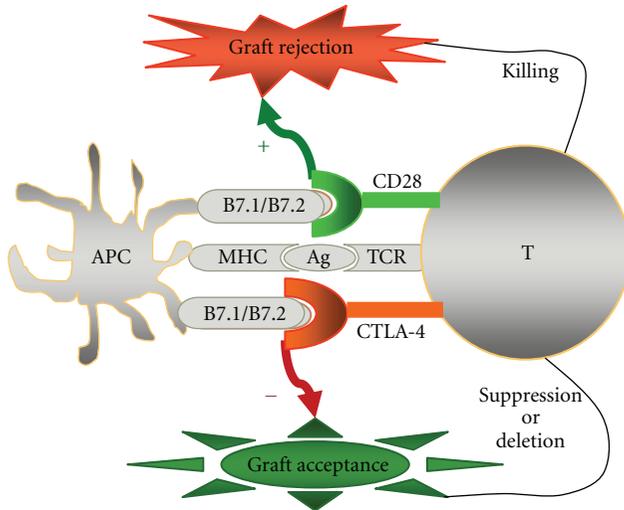


FIGURE 2: The outcome of transplantation is determined by cosignalling. Full T-cell activation requires two signals. The first is antigen-specific recognition of TCR and alloantigen presented on MHC. The second is costimulation which can be positive or negative. In the presence of positive cosignalling (such as with CD28), T-cell proliferation occurs. These activated T cells attack the donor graft and results in graft rejection. One the other hand, the activated T-cell response is terminated in the presence of negative co-signalling (such as with CTLA-4) which protects the graft from destruction.

B7-H4 are located on chromosomes 1 and 3, respectively. Genomic DNA of B7-H4 consists of 6 exons and 5 introns, and mature protein is encoded by an 849-bp region spanning exons III, IV, and part of V. In both genes, exons I and II form a signal peptide, and V encodes transmembrane and intracellular regions.

The extracellular region of B7-H4 contains one Ig V and one Ig C domain. Within the extracellular domain, mouse B7-H4 shares 90% and 99% amino acid (aa) sequence identity with human and rat B7-H4, respectively, suggesting a conserved identity among species. Although it shares only 21–29% aa sequence identity with B7.1, B7.2, B7-H1, B7-H2, B7-H3, and PD-L2 [35], B7-H4 exhibits a similar overall structure with other members in the B7 family.

B7-H4 mRNA is ubiquitously expressed in both lymphoid and nonlymphoid tissues, including placenta, kidney, liver, lung, ovary, testis, and spleen, suggesting that it plays a potential role in peripheral tissues [35–37]. The mature protein is a 50- to -80-kDa glycosylated molecule with a 28-kDa protein core, and its expression appears to be restricted, suggesting posttranscriptional/translational regulation. B7-H4 expression is induced on mitogen- or LPS-activated B cells, T cells, dendritic cells, monocytes, and macrophages [35–37].

The Ig V region is responsible for the binding with the counter-receptor. In B7.1 and B7.2, the V region contains a conserved strand of A'GFCC'C for CTLA-4/CD28 binding. B7-H4 contains no such conserved face. In fact, B7-H4 binds

to a receptor on activated T cells but not to CTLA-4, ICOS, or PD-1, according to FACS analysis of a B7-H4-transfected 293 cell line [35–37]. B7-H4 is expressed on wild type (WT) but not on B- and T-lymphocyte-attenuator- (BTLA-) deficient cells, suggesting a possible role of BTLA as a B7-H4 receptor [37]. However, recent studies have indicated that BTLA does not bind to B7-H4 directly, and that herpes virus entry mediator (HVEM) may be the unique BTLA ligand [38]. The receptor for B7-H4 is still unknown.

The primary function of B7-H4 appears to downregulate the T-cell response. Several lines of evidence from both *in vitro* and *in vivo* data support this notion. B7-H4 inhibits normal mouse and human TCR-induced T-cell proliferation, cytokine production, and cytotoxicity *in vitro* [35–37]. Similarly, the proliferation of T cells and the secretion of IFN- γ activated by autoantigens (insulin, GAD, and IA-2) from human T1D patients were suppressed in the presence of B7-H4. In addition, ectopic expression of B7-H4 in human β cells also protects these cells from cytotoxicity induced by β -cell antigen-specific T-cell clones derived from T1D patients [39]. The negative effects of B7-H4 on T cells are also confirmed in several *in vivo* systems. Administration of B7-H4.Ig impairs CTL activity in a mouse graft-versus-host disease (GVHD) model [35]. In concordance with this inhibitory effect, administration of monoclonal antibody that blocks endogenous B7-H4 expression increases T-cell proliferation and IL-2 production [35]. Similarly, the injection of a blocking mAb against B7-H4 promotes T-cell responses and exacerbates experimental autoimmune encephalomyelitis (EAE) [36]. Collectively, these results suggest that B7-H4 is a novel negative regulator in the B7 family.

It is not clearly understood how B7-H4 affects the immune responses. Studies into the mechanisms by which B7-H4 regulates T-cell immunity show that the suppressive activity of APCs is associated with expression of B7-H4 *in vitro* [40]. B7-H4 expression on APCs is triggered by Tregs through production of IL-10. Blockade of B7-H4 reduces the suppressive activity mediated by Treg conditioned APCs. In addition, Tregs exhibit suppressive activity by stimulating B7-H4 expression through IL-10. Furthermore, the high expression level of B7-H4 in various cancer cells suggests that it may help those tumors escape immune surveillance [41]. Primary ovarian tumour cells express intracellular B7-H4, whereas only a fraction of tumour macrophages express surface B7-H4. B7-H4⁺ tumour macrophages, but not primary ovarian tumour cells, suppress tumour-associated antigen-specific T-cell immunity. IL-6 and IL-10 are highly expressed in the tumour environment, and they trigger macrophage B7-H4 expression [42]. Collectively, these results suggest that a collaborative interaction between B7-H4 and Tregs may downregulate T-cell immunity.

B7-H4 deficient mice display normal responses to several types of airway inflammation, and they show augmented Th1 responses, suggesting a preferential regulation on Th1 subsets [43]. Interestingly, a subsequent study revealed that B7-H4 suppresses neutrophil-mediated immune responses, indicating its role in innate immunity [44].

6. B7-H4 and Transplantation

As described earlier, B7-H4 inhibits alloreactive CTL activity in mouse GVHD and extends the survival of mice with GVHD [35]. The role of B7-H4 in islet transplantation was first investigated by our group [45]. Local expression of B7-H4 by a recombinant adenovirus (Ad-B7-H4) promotes islet survival in a fully MHC-mismatched mouse model (from BALB/c to C56BL/6) [45]. This result has subsequently been confirmed by Yuan's group, using administration of a B7-H4-transfected NIT cell line into diabetic C57BL/6 mice [46]. We showed that survival is associated with reduced CD8⁺ T cells, preserved β -cell function, and upregulation of Foxp3⁺ in the allograft. Yuan's group showed a reduced amount of IFN- γ and increased Tregs in the spleen of B7-H4-treated recipients. Moreover, we used a secondary transplantation model to demonstrate that B7-H4 not only promotes allograft survival but also induces donor-specific tolerance [45, 47].

Alloreactive responses can be modulated through either deletional or nondeletional mechanisms. B7-H4 controls alloreactive responses through downregulating cytotoxic CD8⁺ subsets in the early stage, suggesting a deletional mechanism in tolerance induction [45]. Furthermore, transcription of IFN- γ and granzyme B were significantly decreased in Ad-B7-H4-treated allografts, suggesting that B7-H4 preferentially inhibits Th1 and CTL locally [47]. The inhibition of Th1 response by B7-H4 is also demonstrated in B7-H4-deficient mice which exhibit augmented expression level of both IFN- γ and T-bet in response to *Leishmania major* infection [43]. Whether a reduced amount of CD8⁺ is associated with Th1-related cytokines, or that low levels of IFN- γ results in a reduced CD8 population has not been investigated. This finding demonstrates that functional allograft is preserved by B7-H4-mediated negative co-signalling which limits both helper CD4⁺ cells towards Th1 formation and cytotoxic CD8⁺ cell towards killing donor tissues.

Apart from deletion-mediated tolerance induction, suppression can also be considered a nondeletional mechanism to modulate alloreactive T-cell responses. In the long term, B7-H4 preferentially upregulates the number of Foxp3 in the allograft and Tregs in the periphery, suggesting the involvement of a suppressive mechanism for allograft survival [45, 47]. The outcome of the alloreactive immune response is determined by the balance of effector and regulatory T cells in the process of priming naïve CD4⁺ T cells in response to alloantigen stimulation. The development of Th1/Th2 effectors often dominates alloreactive T-cell responses, whereas the conversion of Foxp3⁺ from naïve or effector T cells occurs only in the presence of specific stimuli. Studies into the relationship between helper and regulatory T-cell subsets reveal that the development of Foxp3⁺ in the periphery is profoundly inhibited by Th1/Th2 activities [48]. Cytokines for Th1/Th2 polarization, such as IFN- γ , IL-12, and IL-4, inhibit Treg differentiation from naïve cells induced by TGF- β 1, whereas blocking of IFN- γ and/or IL-4 could promote Foxp3⁺ Treg differentiation both *in vitro* and *in vivo* [48]. Improved allograft survival in B7-H4-treated recipients may be due to the well-controlled alloreactive T-cell proliferation

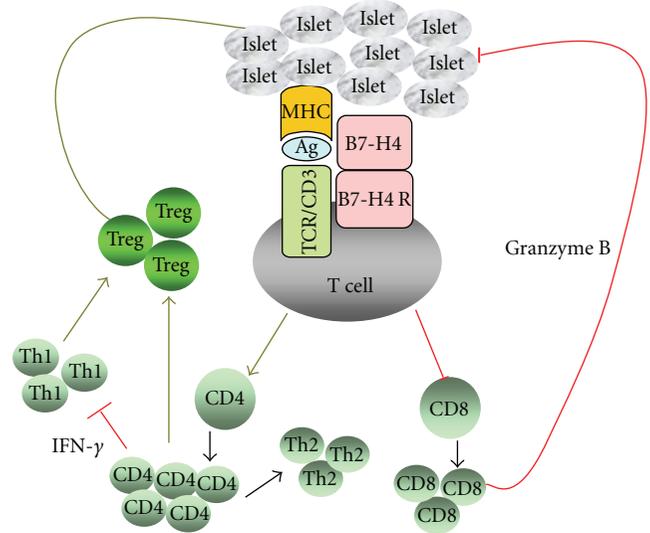


FIGURE 3: The mechanism of B7-H4 action in islet transplantation. Interaction between B7-H4 and an unknown receptor on activated T cells leads to a series of intracellular signalling cascades that result in improved allograft survival. B7-H4 protects allograft rejection through deletional and nondeletional mechanisms. It can limit cytotoxic CD8⁺ proliferation and transcription of granzyme B. It also reduces the Th1 response which may facilitate generation of suppressive Foxp3⁺ Tregs. The combination of controlling activated T-cell proliferation and promoting regulatory T-cell subsets results in enhanced allograft survival and induction of donor-specific tolerance.

suppressed by increased number of Foxp3⁺. Reduced IFN- γ in the early stage may facilitate the conversion of Foxp3⁺ from effector or naïve T cells in the late stage.

B7-H4 enhances islet allograft survival and induces donor-specific tolerance through deletional and non-deletional mechanisms (Figure 3). However, the mechanism for tolerance induction and maintenance is not fully understood. A similar number of Tregs in the periphery of failed and surviving recipients treated with B7-H4 after second-set transplants demonstrated that factors other than Tregs may contribute to tolerance maintenance, such as inefficient inhibition of memory T cells generated in second-set transplants [47]. Theoretically, iTregs are supposed to be donor-specific because they are converted from effector cells that share a similar TCR repertoire. Therefore, the generation of iTregs might result in donor-specific tolerance. Epigenetic studies reveal that CpG dinucleotides at the Foxp3 locus are methylated in naïve CD4⁺CD25⁻, activated CD4⁺ T cells, and TGF- β -induced iTregs but completely demethylated in nTregs, demonstrating closely compact nucleosomes in the formal subsets that prevent transcription [49]. In fact, iTregs are particularly unstable and tend to lose Foxp3 expression more easily than nTregs. In B7-H4-treated recipients, suppressive Tregs and cytopathogenic Teffs can be reprogrammed upon secondary donor-specific antigen stimulation, indicating that Tregs are involved in tolerance induction but play a minimal role in tolerance maintenance. The relative contributions of nTregs and iTregs in terms of

synergism in promoting tolerance or redundant remain to be elucidated.

7. B7-H4 and Autoimmunity

Mice treated with B7-H4-neutralizing mAb that blocks endogenous B7-H4 expression develop accelerated and much more robust EAE, demonstrating that B7-H4 downregulates autoimmune EAE [36]. Moreover, regulation of myelin oligodendrocyte glycoprotein- (MOG-) induced EAE by B7-H4-blocking mAb is associated with increased CD4⁺, CD8⁺, and CD11b⁺ macrophages, suggesting its role in modulating the interaction between T cells and APCs in EAE [36].

Our unpublished data show that early treatment of NOD mice with B7-H4.Ig fusion protein significantly reduces the incidence of spontaneous autoimmune disease to 28.6%, compared to 67.8% in controls, suggesting its efficacy in preventing the destruction of insulin-producing β cells by autoreactive T cells. These promising data demonstrate B7-H4's potential role in inhibiting recurrence of autoimmune diabetes in islet transplantation.

8. Future Directions and Conclusions

Much progress has been made in improving allograft survival and induction of tolerance using costimulation blockade. CTLA-4.Ig prolongs survival of allograft and xenografts in various rodent models [50–52]. Its mutant form LEA29A (belatacept), which increases the efficacy of inhibition through enhanced binding capacity and decreased dissociation rate with B7.1/B7.2, shows promising results in nonhuman primates and in human renal transplantation and is currently in phase III clinical trials [53, 54]. Programmed cell death ligand 1 (PD-L1) fusion protein, PD-L1.Ig, prolongs cardiac allograft survival in CD28-deficient but not in wild-type recipients, suggesting that PD-1:PD-L1 and CD28/CTLA-4:B7 may be two distinct pathways in alloimmunity [55]. Combination of PDL1.Ig and anti-CD154 prolongs islet allograft survival [56].

In a mouse solid organ (heart) transplantation model, B7-H4 plays a nonredundant regulatory role and functions dominantly in the absence of CD28/CTLA-4:B7 signals, suggesting its synergistic effects with CTLA-4 to control allograft rejection, especially facilitatory to tolerance induction [57]. In order to design new strategies for preventing both alloreactive and autoreactive immune responses and generating donor-specific tolerance, it will be important to identify the detailed mechanisms in each cosignalling pathway.

Abbreviations

APC:	Antigen- presenting cell
TCR:	T-cell receptor
EST:	Expressed sequence tagged
MHC:	Major histocompatibility complex
HLA:	Human leukocyte antigen
HbA1C:	Hemoglobin A1C
IL:	Interleukin
OVA:	Ovalbumin.

Conflict of Interests

The authors declare no conflict of interest.

Acknowledgments

This project was funded by the Juvenile Diabetes Research Foundation, the Canadian Institutes of Health Research (MOP-79414), and the Michael Smith Foundation for Health Research. Xiaojie Wang is a recipient of fellowships from the CIHR/Michael Smith Foundation for Health Research Strategic Training Program in Transplantation Research and the University of British Columbia Graduate program. C. Bruce Verchere is a Michael Smith Foundation for Health Research Senior Scholar.

References

- [1] C. Jahansouz, S. C. Kumer, M. Ellenbogen, and K. L. Brayman, "Evolution of β -cell replacement therapy in diabetes mellitus: pancreas transplantation," *Diabetes Technology & Therapeutics*, vol. 13, no. 3, pp. 395–418, 2011.
- [2] M. A. Fung, G. L. Warnock, Z. Ao et al., "The effect of medical therapy and islet cell transplantation on diabetic nephropathy: an interim report," *Transplantation*, vol. 84, no. 1, pp. 17–22, 2007.
- [3] G. L. Warnock, R. M. Meloche, D. Thompson et al., "Improved human pancreatic islet isolation for a prospective cohort study of islet transplantation vs best medical therapy in type 1 diabetes mellitus," *Archives of Surgery*, vol. 140, no. 8, pp. 735–744, 2005.
- [4] T. L. van Belle, K. T. Coppieters, and M. G. von Herrath, "Type 1 diabetes: etiology, immunology, and therapeutic strategies," *Physiological Reviews*, vol. 91, no. 1, pp. 79–118, 2011.
- [5] G. L. Warnock, D. M. Thompson, R. M. Meloche et al., "A multi-year analysis of islet transplantation compared with intensive medical therapy on progression of complications in type 1 diabetes," *Transplantation*, vol. 86, no. 12, pp. 1762–1766, 2008.
- [6] G. L. Warnock, Y. H. T. Liao, X. Wang et al., "An odyssey of islet transplantation for therapy of type 1 diabetes," *World Journal of Surgery*, vol. 31, no. 8, pp. 1569–1576, 2007.
- [7] A. M. J. Shapiro, J. R. T. Lakey, E. A. Ryan et al., "Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen," *The New England Journal of Medicine*, vol. 343, no. 4, pp. 230–238, 2000.
- [8] A. M. J. Shapiro, C. Ricordi, B. J. Hering et al., "International trial of the Edmonton protocol for islet transplantation," *The New England Journal of Medicine*, vol. 355, no. 13, pp. 1318–1330, 2006.
- [9] D. M. Thompson, M. Meloche, Z. Ao et al., "Reduced progression of diabetic microvascular complications with islet cell transplantation compared with intensive medical therapy," *Transplantation*, vol. 91, no. 3, pp. 373–378, 2011.
- [10] V. Yechoor and L. Chan, "Minireview: β -cell replacement therapy for diabetes in the 21st century: manipulation of cell fate by directed differentiation," *Molecular Endocrinology*, vol. 24, no. 8, pp. 1501–1511, 2010.
- [11] M. Borowiak and D. A. Melton, "How to make β cells?" *Current Opinion in Cell Biology*, vol. 21, no. 6, pp. 727–732, 2009.

- [12] J. D. Johnson, Z. Ao, P. Ao et al., "Different effects of FK506, rapamycin, and mycophenolate mofetil on glucose-stimulated insulin release and apoptosis in human islets," *Cell Transplantation*, vol. 18, no. 8, pp. 833–845, 2009.
- [13] B. Afzali, G. Lombardi, and R. I. Lechler, "Pathways of major histocompatibility complex allorecognition," *Current Opinion in Organ Transplantation*, vol. 13, no. 4, pp. 438–444, 2008.
- [14] F. Issa, A. Schiopu, and K. J. Wood, "Role of T cells in graft rejection and transplantation tolerance," *Expert Review of Clinical Immunology*, vol. 6, no. 1, pp. 155–169, 2010.
- [15] N. A. Mitchison, "Adoptive transfer of immune reactions by cells," *Journal of Cellular Physiology*, vol. 50, no. 1, Supplement 1, pp. 247–264, 1957.
- [16] N. A. Mitchison, "Studies on the immunological response to foreign tumor transplants in the mouse. I. The role of lymph node cells in conferring immunity by adoptive transfer," *The Journal of Experimental Medicine*, vol. 102, no. 2, pp. 157–177, 1955.
- [17] J. A. Shizuru, A. K. Gregory, C. T. B. Chao, and C. G. Fathman, "Islet allograft survival after a single course of treatment of recipient with antibody to L3T4," *Science*, vol. 237, no. 4812, pp. 278–280, 1987.
- [18] K. Seydel, J. Shizuru, D. Grossman, A. Wu, S. Alters, and C. G. Fathman, "Anti-CD8 abrogates effect of anti-CD4-mediated islet allograft survival in rat model," *Diabetes*, vol. 40, no. 11, pp. 1430–1434, 1991.
- [19] R. G. Gill, A. S. Rosenberg, K. J. Lafferty, and A. Singer, "Characterization of primary T cell subsets mediating rejection of pancreatic islet grafts," *Journal of Immunology*, vol. 143, no. 7, pp. 2176–2178, 1989.
- [20] P. Nickerson, W. Steurer, J. Steiger, X. Zheng, A. W. Steele, and T. B. Strom, "Cytokines and the Th1/Th2 paradigm in transplantation," *Current Opinion in Immunology*, vol. 6, no. 5, pp. 757–764, 1994.
- [21] E. M. Shevach, R. S. McHugh, C. A. Piccirillo, and A. M. Thornton, "Control of T-cell activation by CD4+ CD25+ suppressor T cells," *Immunological Reviews*, vol. 182, pp. 58–67, 2001.
- [22] P. T. Walsh, D. K. Taylor, and L. A. Turka, "Tregs and transplantation tolerance," *Journal of Clinical Investigation*, vol. 114, no. 10, pp. 1398–1403, 2004.
- [23] S. Sakaguchi, N. Sakaguchi, M. Asano, M. Itoh, and M. Toda, "Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α -chains (CD25): breakdown of a single mechanism of self-tolerance causes various autoimmune diseases," *Journal of Immunology*, vol. 155, no. 3, pp. 1151–1164, 1995.
- [24] H. Jonuleit and E. Schmitt, "The regulator T cell family: distinct subsets and their interrelations," *Journal of Immunology*, vol. 171, no. 12, pp. 6323–6327, 2003.
- [25] P. A. Taylor, R. J. Noelle, and B. R. Blazar, "CD4+CD25+ immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade," *Journal of Experimental Medicine*, vol. 193, no. 11, pp. 1311–1317, 2001.
- [26] H. Groux, A. O'Garra, M. Bigler et al., "A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis," *Nature*, vol. 389, no. 6652, pp. 737–742, 1997.
- [27] M. G. Roncarolo, M. K. Levings, and C. Traversari, "Differentiation of T regulatory cells by immature dendritic cells," *Journal of Experimental Medicine*, vol. 193, no. 2, pp. F5–F9, 2001.
- [28] L. Graca, S. P. Cobbold, and H. Waldmann, "Identification of regulatory T cells in tolerated allografts," *Journal of Experimental Medicine*, vol. 195, no. 12, pp. 1641–1646, 2002.
- [29] Y. Chen, V. K. Kuchroo, J. Inobe, D. A. Hafler, and H. L. Weiner, "Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis," *Science*, vol. 265, no. 5176, pp. 1237–1240, 1994.
- [30] W. Chen, W. Jin, N. Hardegen et al., "Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF- β induction of transcription factor Foxp3," *Journal of Experimental Medicine*, vol. 198, no. 12, pp. 1875–1886, 2003.
- [31] M. Karim, C. I. Kingsley, A. R. Bushell, B. S. Sawitzki, and K. J. Wood, "Alloantigen-induced CD25+CD4+ regulatory T cells can develop in vivo from CD25-CD4+ precursors in a thymus-independent process," *Journal of Immunology*, vol. 172, no. 2, pp. 923–928, 2004.
- [32] M. K. Jenkins, J. D. Ashwell, and R. H. Schwartz, "Allogeneic non-T spleen cells restore the responsiveness of normal T cell clones stimulated with antigen and chemically modified antigen-presenting cells," *Journal of Immunology*, vol. 140, no. 10, pp. 3324–3330, 1988.
- [33] M. F. Krummel and J. P. Allison, "CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation," *Journal of Experimental Medicine*, vol. 182, no. 2, pp. 459–465, 1995.
- [34] R. H. Schwartz, "A cell culture model for T lymphocyte clonal anergy," *Science*, vol. 248, no. 4961, pp. 1349–1356, 1990.
- [35] G. L. Sica, I. H. Choi, G. Zhu et al., "B7-H4, a molecule of the B7 family, negatively regulates T cell immunity," *Immunity*, vol. 18, no. 6, pp. 849–861, 2003.
- [36] D. V. R. Prasad, S. Richards, X. M. Mai, and C. Dong, "B7S1, a novel B7 family member that negatively regulates T cell activation," *Immunity*, vol. 18, no. 6, pp. 863–873, 2003.
- [37] X. Zang, P. Loke, J. Kim, K. Murphy, R. Waitz, and J. P. Allison, "B7x: a widely expressed B7 family member that inhibits T cell activation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 18, pp. 10388–10392, 2003.
- [38] J. R. Sedy, M. Gavrieli, K. G. Potter et al., "B and T lymphocyte attenuator regulates T cell activation through interaction with herpesvirus entry mediator," *Nature Immunology*, vol. 6, no. 1, pp. 90–98, 2005.
- [39] D. Ou, X. Wang, D. L. Metzger et al., "Suppression of human T-cell responses to β -cells by activation of B7-H4 pathway," *Cell Transplantation*, vol. 15, no. 5, pp. 399–410, 2006.
- [40] I. Kryczek, S. Wei, L. Zou et al., "Cutting edge: induction of B7-H4 on APCs through IL-10: novel suppressive mode for regulatory T cells," *Journal of Immunology*, vol. 177, no. 1, pp. 40–44, 2006.
- [41] I. Kryczek, L. Zou, P. Rodriguez et al., "B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma," *Journal of Experimental Medicine*, vol. 203, no. 4, pp. 871–881, 2006.
- [42] I. Kryczek, S. Wei, G. Zhu et al., "Relationship between B7-H4, regulatory T cells, and patient outcome in human ovarian carcinoma," *Cancer Research*, vol. 67, no. 18, pp. 8900–8905, 2007.
- [43] W. K. Suh, S. Wang, G. S. Duncan et al., "Generation and characterization of B7-H4/B7S1/B7x-deficient mice," *Molecular and Cellular Biology*, vol. 26, no. 17, pp. 6403–6411, 2006.
- [44] G. Zhu, M. M. Augustine, T. Azuma et al., "B7-H4-deficient mice display augmented neutrophil-mediated innate immunity," *Blood*, vol. 113, no. 8, pp. 1759–1767, 2009.
- [45] X. Wang, J. Hao, D. L. Metzger et al., "Local expression of B7-H4 by recombinant adenovirus transduction in mouse islets prolongs allograft survival," *Transplantation*, vol. 87, no. 4, pp. 482–490, 2009.

- [46] C. L. Yuan, J. F. Xu, J. Tong et al., "B7-H4 transfection prolongs β -cell graft survival," *Transplant Immunology*, vol. 21, no. 3, pp. 143–149, 2009.
- [47] X. Wang, J. Hao, D. L. Metzger et al., "B7-H4 induces donor specific tolerance in mouse allografts," *Cell Transplantation*. In press.
- [48] J. Wei, O. Duramad, O. A. Perng, S. L. Reiner, Y. J. Liu, and F. X. F. Qin, "Antagonistic nature of T helper 1/2 developmental programs in opposing peripheral induction of Foxp3+ regulatory T cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 46, pp. 18169–18174, 2007.
- [49] G. Lal and J. S. Bromberg, "Epigenetic mechanisms of regulation of Foxp3 expression," *Blood*, vol. 114, no. 18, pp. 3727–3735, 2009.
- [50] D. J. Lenschow, Y. Zeng, J. R. Thistlethwaite et al., "Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4Ig," *Science*, vol. 257, no. 5071, pp. 789–792, 1992.
- [51] P. Y. Benhamou, "Immunomodulation with CTLA4-Ig in islet transplantation," *Transplantation*, vol. 73, no. 1, pp. S40–S42, 2002.
- [52] A. B. Adams, N. Shirasugi, T. R. Jones et al., "Development of a chimeric anti-CD40 monoclonal antibody that synergizes with LEA29Y to prolong islet allograft survival," *Journal of Immunology*, vol. 174, no. 1, pp. 542–550, 2005.
- [53] F. Vincenti, C. Larsen, A. Durrbach et al., "Costimulation blockade with belatacept in renal transplantation," *The New England Journal of Medicine*, vol. 353, no. 8, pp. 770–781, 2005.
- [54] Y. Vanrenterghem, B. Bresnahan, J. Campistol et al., "Belatacept-based regimens are associated with improved cardiovascular and metabolic risk factors compared with cyclosporine in kidney transplant recipients (BENEFIT and BENEFIT-EXT studies)," *Transplantation*, vol. 91, no. 9, pp. 976–983, 2011.
- [55] E. Özkaynak, L. Wang, A. Goodearl et al., "Programmed death-1 targeting can promote allograft survival," *Journal of Immunology*, vol. 169, no. 11, pp. 6546–6553, 2002.
- [56] W. Gao, G. Demirci, T. B. Strom, and X. C. Li, "Stimulating PD-1-negative signals concurrent with blocking CD154 co-stimulation induces long-term islet allograft survival," *Transplantation*, vol. 76, no. 6, pp. 994–999, 2003.
- [57] K. Yamaura, T. Watanabe, O. Boenisch et al., "In vivo function of immune inhibitory molecule B7-H4 in alloimmune responses: brief communication," *American Journal of Transplantation*, vol. 10, no. 10, pp. 2355–2362, 2010.

Review Article

β -Cell Generation: Can Rodent Studies Be Translated to Humans?

Françoise Carlotti,¹ Arnaud Zaldumbide,² Johanne H. Ellenbroek,¹ H. Siebe Spijker,¹
Rob C. Hoeben,² and Eelco J. de Koning^{1,3}

¹ Department of Nephrology, Leiden University Medical Center, Postal Zone C3-P, P.O. Box 9600, 2300 RC Leiden, The Netherlands

² Department of Molecular Cell Biology, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands

³ Hubrecht Institute, 3584 CX Utrecht, The Netherlands

Correspondence should be addressed to Françoise Carlotti, f.carlotti@lumc.nl

Received 31 May 2011; Revised 31 July 2011; Accepted 31 July 2011

Academic Editor: Thierry Berney

Copyright © 2011 Françoise Carlotti et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

β -cell replacement by allogeneic islet transplantation is a promising approach for patients with type 1 diabetes, but the shortage of organ donors requires new sources of β cells. Islet regeneration *in vivo* and generation of β -cells *ex vivo* followed by transplantation represent attractive therapeutic alternatives to restore the β -cell mass. In this paper, we discuss different postnatal cell types that have been envisaged as potential sources for future β -cell replacement therapy. The ultimate goal being translation to the clinic, a particular attention is given to the discrepancies between findings from studies performed in rodents (both *ex vivo* on primary cells and *in vivo* on animal models), when compared with clinical data and studies performed on human cells.

1. Introduction

Type 1 diabetes results from the specific destruction of β -cells by the immune system. The insulin-producing β -cells are the most abundant cell type residing in the islets of Langerhans, which are microorgans that are scattered throughout the exocrine tissue and represent only 1 to 2% of the total organ mass. β -cell replacement is considered the best therapeutic option, given the capacity of this particular cell type to accurately respond to highly variable changes in blood glucose level and as such to maintain glucose homeostasis. Islet transplantation via the portal vein of the liver is a promising approach and considerably less invasive than total organ transplantation. However, among other difficulties, the scarcity of donor material will always remain a major hurdle [1, 2].

Adult β -cell mass is known to be dynamic and to be able to respond to physiological changes in insulin demand such as obesity, pregnancy, and starvation. In theory, β -cell mass adaptation can occur via modification of cell size (hypertrophy versus atrophy) or modification of cell number (proliferation of existing mature β -cells or formation of new β -cells from progenitor cells versus apoptosis).

The origin of newly formed β -cells has long been debated [3–9], part of the observations reported remaining controversial and partial. In addition, many statements rely on studies performed in animal models or animal cell(s) (lines). As the ultimate goal is translation to the clinic, the aim of this paper will be to compare rodent and human data regarding postnatal cell types envisaged as potential sources to generate β -cells during the past decade.

We will focus on the replication capacity of the β -cell itself then address the unexpected recent plasticity of differentiated cell types developmentally close to β -cells. For many years the dogma was upheld that terminally differentiated cells were committed to a specific function and could no longer change their identity. In contrast, progenitor/stem cells are expected to retain some multipotency capacities and therefore be more suitable for tissue replacement strategies. Nowadays more and more examples of efficient “transdifferentiation” have been reported, without an apparent need for a progenitor cell intermediate stage. Finally, given their various capacities, adult mesenchymal stem cells (MSC) are increasingly considered for clinical use in many different applications. We will review the recent reports about both pancreatic and extrapancreatic sources of

MSC and the possible application of these cells for type 1 diabetes treatment.

Several groups reported on a successful derivation of both murine and human embryonic stem cells (ESs) to β -cell-like cells able to revert hyperglycemia in diabetic mouse models (for detailed review see [10]). Mimicking normal pancreatogenesis appears to be the best strategy to differentiate ES cells toward the endocrine lineage, although large differences in differentiation capacity exist between different ES cell lines using similar protocols [11]. Importantly, the risk of teratomas formation *in vivo*, inherent to ES cells when they remain undifferentiated, as well as ethical principles about any therapeutical use of human ES cells currently hold back extensive clinical applications of these cells. Recent developments on technology to generate induced pluripotent stem cells (iPS) hold great promise [12–16]. However, safety issues due to genetic and epigenetic abnormalities during reprogramming or in subsequent cell culture are a major hurdle for clinical transplantation. A more immediate application of ES and iPS cells is more likely to be their use in modeling human disease [17]. Therefore, in this paper, we chose to limit ourselves to alternative postnatal cell sources. The data of this part are summarized in Table 1.

2. Generation of β -Cells from Mature Differentiated Cells?

2.1. β -cells. During adult life, β -cell replication appears to be a predominant mechanism of β -cell mass expansion in healthy mice (Figure 1). Dor et al. demonstrated that preexisting β -cells, rather than multipotent stem cells, are the major source of new β -cells during adult life and after partial pancreatectomy [18]. The study relies on a transgenic mouse model carrying a rat insulin gene promoter-controlled expression cassette encoding a tamoxifen-dependent CRE recombinase. A pulse treatment of tamoxifen irreversibly induced the expression of a reporter gene (human placental alkaline phosphatase (HPAP)) through CRE-mediated excision of a STOP sequence specifically in β -cells. Intriguingly, the percentage of HPAP-positive β -cells immediately after tamoxifen treatment and 4, 6, 9, and 12 months later remained very similar, indicating that during normal turnover in murine β -cells originate exclusively from preexisting β -cells. In islet injury models, either by pancreatectomy or by β -cell ablation using β -cell specific expression of Diphtheria toxin A resulting in 70% and 80% reduction in β -cell mass, respectively [18, 19], new β -cells appeared to be formed largely from genetically labeled preexisting β -cells and not from neogenesis or from expansion of non- β -cell precursors. Nevertheless, murine β -cell replication capacity appears to decline with age [20–22]. Furthermore, the question was addressed whether all β -cells contribute equally to growth and maintenance of β -cell mass or if distinct subpopulations exist. Label-retaining techniques were applied. Brennand et al. performed an *in vivo* pulse-chase labeling experiment using Histone 2B-GFP as reporter gene [23]. A uniform label across the entire β -cell population was observed. Next, a clonal analysis of dividing β -cells

TABLE 1: Potential sources of de novo β -cells among differentiated adult cell types.

	Rodent		Human	
	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>
<i>Endocrine</i>				
β -cells	+++	+++	–	–/+
α -cells	?	++	?	?
<i>Exocrine</i>				
Ductal cells	++	+	+	++
Acinar cells	+	–/+	?	?
<i>Extra-pancreatic</i>				
Liver cells	?	++	+	?

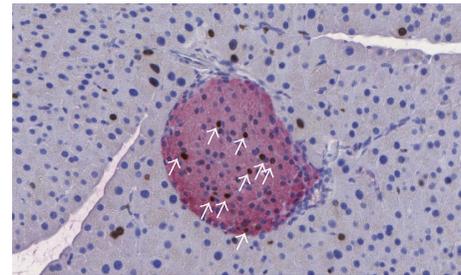


FIGURE 1: Section of murine pancreas stained with anti-insulin (red) and anti-BrdU (brown) antibodies. C57BL/6 mice were on a high-fat diet for 6 weeks. BrdU was injected for one week by s.c. injections, thereby labeling all proliferating cells. A number of replicating β -cells (arrows) and acinar cells are detected.

revealed that all clones were of similar sizes. Altogether this suggests that, in mice, the pool of β -cells is homogenous and all cells are able to replicate at the same rate. Therefore all β -cells can be candidate for *in vitro* expansion. Similar conclusions were obtained from a parallel study, in which a DNA analog-based lineage tracing method was developed to detect sequential cell division *in vivo* [24]. Recently Dor and colleagues further investigated the replication dynamics of adult murine β -cells and showed that replicated β -cells are able to reenter the cell division cycle shortly after mitosis. This short quiescence period of several days was found to be lengthened with advanced age [25] and shortened during injury-driven β -cell regeneration and following treatment with a pharmacological activator of glucokinase [25, 26]. The data of this part are summarized in Table 1.

In humans, Meier et al. investigated the β -cell mass from infancy to adulthood by performing immunohistochemistry and morphometric analyses on pancreas obtained at autopsy from 46 donors (from 2 weeks to 21 years of age) and determined pancreas volume by CT scan in 135 individuals of similar age [27]. The authors concluded that the predominant expansion of β -cell mass in humans occurs in early childhood (2.6% of β -cells positive for Ki67), without secondary growth phase of β -cell mass during adolescence, in contrast to rodents. Interestingly, the islets appear to increase in size rather than in number. Moreover, although the authors identified some β -cells (both individual cells

and in small clusters) near the ducts, the number of these insulin-positive cells increased to a similar extent as the overall expansion of β -cell numbers in the islets, consistent with the hypothesis of β -cell replication. It is important to note that an efficient β -cell mass regeneration approach in a therapeutic perspective would require a similar rate of β -cell replication. Other studies confirmed these results. Kassem et al. reported that β -cell replication decreased progressively from 3.2% at 17–32 weeks of gestation to 1.1% perinatally [28]. After birth, levels of β -cell replication would drop further to reach less than 0.1% in young adults [29]. These data also correlate with the findings that β -cell mass is established by the first two or three decades of human life as determined by measuring accumulation of lipofuscin bodies as a marker to estimate β -cell longevity [30] or by *in vivo* thymidine analog incorporation and radiocarbon dating [31]. Remarkably, the adaptive increase of β -cell mass in adult humans appears to be modest in response to obesity (2- versus 10-fold) [32], as well as during pregnancy (1.4 fold versus 2 to 5-fold) compared to rodents [33, 34]. In contrast to rodents again, in both obese subjects and pregnant women, the adaptive increase in β -cell number was accompanied by an increased number of small new islets, indicative of neogenesis (cf. paragraph 2.3), rather than an increase in islet size or number of β -cells per islet, characteristic for β -cell replication. Finally a study performed on human pancreatic tissue collected from 13 patients who underwent a partial pancreatectomy showed that, unlike in rodents, a 50% pancreatectomy does not trigger any β -cell regeneration in adult humans. This corroborates with the high incidence of diabetes after partial pancreatectomy [35]. In summary, *in vivo* β -cell replication capacity in humans appears to be mostly limited to the very early postnatal period, and the triggers of such a process are still unknown.

Data available on β -cell proliferation *in vitro* are very limited when restricted to primary β -cells. An early report claimed that human β -cells were able to replicate efficiently (69% of BrdU/insulin double-positive cells) when exposed to a specific matrix (matrix produced by the rat bladder carcinoma cell line 804G) in the presence of hepatocyte growth factor/scatter factor (HGF/SF) [36]. However HGF induced a rapid decrease in insulin content [37]. This work was rapidly challenged by another report showing that the defined culture conditions were favorable for replication of ductal cells and not for β -cells [38]. In a comparative study between *in vitro* proliferation of purified human and rat β -cells, Parnaud et al. also failed to detect any β -cells that were positive for Ki67 or had incorporated BrdU even after 10 days of exposure [39]. However a clear proliferation of purified rat β -cells was observed and could be further enhanced by defined coatings or growth factors. Proliferation of human β -cells in (intact) isolated human islets was also assessed in the same study and remained undetectable. Therefore it appears that culture conditions for efficient human β -cell replication *ex vivo* have not been clearly identified.

2.2. α Cells. Differentiation of endocrine non- β -cells to β -cells is an interesting alternative mechanism for increasing the β -cell mass. A limited number of studies supported this

new concept. Collombat et al. showed in various transgenic mouse models that forced expression of Pax4 at different stages (in pancreatic progenitor cells, endocrine precursor cells, or in mature α cells) resulted in a shift of all endocrine lineages toward a β -cell fate [40]. A bicistronic vector (Pax4-IRES- β -galactosidase) was used in order to follow cells of interest by staining for β -galactosidase activity. Importantly, the authors observed an age-dependent increase in islet size and in number of insulin/ β -galactosidase double-positive cells and a concomitant decrease in α -cell content. These observations indicate that ectopic Pax4 expression can also force conversion of adult glucagon-expressing cells into β -cells. Remarkably, the subsequent decrease in glucagon was found to activate the differentiation of ducts-associated progenitor cells α cells (via an intermediate stage of Ngn3 positive cells). However the newly formed α cells failed to correct the hypoglucagonemia since they were shown to be rapidly converted into β -cells upon Pax4 ectopic expression. Notably, the expression of Pax4 in glucagon-positive cells has been reported to be sufficient to restore a functional β -cell mass in diabetic mice, although only in the young animals. Thorel et al. developed a transgenic mouse model of near total β -cell ablation that relies on the specific expression of Diphtheria Toxin Receptor (DTR) in pancreatic β -cells (expression driven by the rat insulin promoter) [41]. After administration of Diphtheria Toxin, a rapid and extreme (>99%) β -cell destruction by apoptosis was observed resulting in a characteristic diabetes within a couple of weeks. If given insulin, the mice survived and showed a slow β -cell mass regeneration. Only after 5 months the regenerated β -cell mass was able to maintain glucose homeostasis without exogenous insulin administration. Using a doxycycline inducible α -cell lineage tracing system, the authors established that one month after β -cell ablation, a large but variable (32 to 81%) fraction of newly formed β -cells resulted from the transdifferentiation of about 5 to 10% of α cells. Importantly, almost all (~90%) were bihormonal (still expressing glucagon even as far as 10 months after ablation). Of note, no β -cells were found in extrainsular locations. Therefore, in contrast to previous studies [19], the β -cell mass regeneration was attributed to neogenesis through α -cell transdifferentiation, rather than to the slow self-replication mechanism of preexisting mature β -cells. The authors suggested that the amount of β -cell loss and the type of injury would determine the mechanism of regeneration. This theory of unexpected pancreatic cell plasticity triggered by extreme β -cell loss was supported by a parallel study done by Chung and colleagues [42]. The model used was a combination of pancreatic duct ligation (PDL) with specific elimination of preexisting β -cells by alloxan [42]. No lineage tracing method was used in this work, and results are based on immunohistochemistry stainings performed at 7 and 14 days. The authors reported a rapid regeneration of β -cell mass within weeks and suggested that the newly formed β -cells resulted mainly from conversion of adult α cells. Interestingly the authors also observed that under injury conditions, α cells were able to replicate. However no α -cell division was required for conversion into β -cells. Importantly the regenerated β -cell mass was

not sufficient to revert hyperglycemia, possibly due to the persistent inflammation caused by the PDL. In summary, three independent studies performed in transgenic mouse models showed that α - to β -cell conversion can occur. One could wonder how this relates with the developmental process. In rodents, the glucagon gene is expressed in the earliest endocrine cells that can be detected [43]. However, Herrera et al. demonstrated that, during murine embryogenesis, mature glucagon- and insulin-producing cells share a common precursor but belong to separate developmental lineages [44].

In human cells, the embryologic situation appears to be different, since insulin-positive cells are the first to be detected during development [45]. Therefore it is not known whether this extraordinary islet cell plasticity exists in adult human α cells *in vitro* or *in vivo*. If so, α cells, especially if their capacity to replicate under injury condition is confirmed, could be an ideal intraislet source for *in vivo* regeneration of β -cells.

2.3. Ductal Cells. Pancreatic ductal cells have long been thought to be the main source for progenitor cells in the pancreas (cf. reviews [3, 8]).

Several rodent studies based on immunohistochemical observations in animal models, suggested a possible mechanism of islet neogenesis via recapitulation of the embryological development after activation and differentiation of ductal progenitors [46–50]. Recently a number of lineage tracing studies in transgenic mouse models have been performed in order to identify putative endocrine progenitors in adults, but provided contradictory conclusions. Bonner-Weir and collaborators made use of the human carbonic anhydrase II (CAII) promoter, specific for differentiated ductal cells, combined to a tamoxifen inducible CRE-ER/Lox recombination system [51]. CAII-expressing cells within the pancreas acting as precursor cells gave rise to both pancreatic endocrine and exocrine tissues after birth and after injury. After PDL 25% of β -cells were labeled. The authors proposed that ductal cells represent a pool of homogenous cells from which a fraction can dedifferentiate into progenitor cells that are able to regenerate both endocrine and exocrine tissues. In a parallel study, Heimberg and colleagues suggested a slightly different model: a rare subpopulation of endocrine progenitor cells, located in the ductal lining, could be activated upon PDL in adult mouse pancreas and subsequently started to express an embryonic key endocrine developmental factor, Ngn3 [52]. Differentiation of an Ngn3-positive subpopulation gave rise to all islet cell types, including glucose responsive β -cells both *in situ* and when cultured in embryonic pancreatic explants. However, in a subsequent study a lineage tracing of HNF1 β -positive ductal cells was performed. 65% of pancreatic ductal cells were labeled. The authors show that the ductal epithelium does not make a significant contribution to acinar or endocrine cells during neonatal growth (6-month observation period) or upon regeneration condition (PDL or Alloxan followed by EGF/gastrin treatment) [53]. Of interest, earlier lineage studies by Melton's group pointed out on the heterogeneity in developmental potential among "duct-like structures" in early embryo [54].

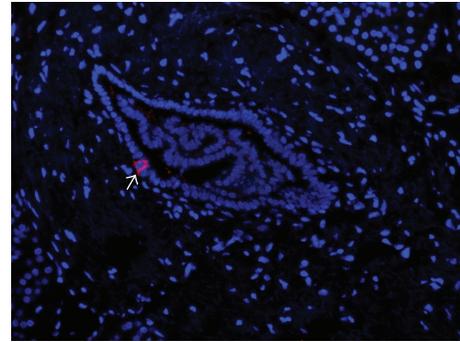


FIGURE 2: Section of human pancreatic exocrine tissue stained with an anti-insulin antibody (red). Nuclei are stained by DAPI (blue). An insulin-positive cell (arrow) is detected in the duct lining suggesting a process of β -cell neogenesis.

Lineage tracing experiments suggested that Ngn3-positive cells are indeed islet progenitors but distinct from duct progenitors. On the other hand, the authors could not rule out the possibility that a minor population of mature ductal cells is able to transiently activate Ngn3 gene expression and subsequently contributes to islets neogenesis. Lineage tracing of Muc1 ductal/acinar cells confirmed that Muc1-positive cells give rise to endocrine cells *in utero*, in line with embryological development [55]. However, after birth, Muc1 lineage-labeled cells were found to be restricted to the exocrine compartment, with no detectable contribution to islet cells. No injury or regeneration models have been tested in that particular model. Along the same line, another independent lineage labeling using the Sox9 promoter (active in early pancreatic progenitors) and resulting in 70% of labeled pancreatic ductal cells established that very few nonendocrine cells continue to arise from Sox9-positive precursors in early postnatal life, but no endocrine or acinar cell neogenesis from Sox9-positive cells occurs during adulthood [56]. Intriguingly, in contrast with an earlier study [52], the authors found that after PDL, Sox9-positive cells give rise to Ngn3-positive cells but these cells do not contribute to islets. Thus, although the function of ductal cells as endocrine progenitors in embryonic development is commonly accepted, more insight is needed into the potential of cells from the ductal compartment as an alternative source for the formation of new β -cells in adult rodents.

Immunohistochemistry stainings performed on tissues obtained at autopsy or after pancreatectomy are obviously the only possible technique to evaluate the β -cell mass in humans. The occurrence of neogenesis is suggested by the presence of insulin-positive cells in the ductal area combined to the absence of replication of this cell population (ruling out the expansion of a preexisting insulin-positive ductal cell population) (Figure 2). Careful quantification and repeated observations in different contexts can result in strong indications. Meier et al. analyzed pancreatic tissues obtained at autopsy from 46 children aged from 2 weeks to 21 years and observed about 0.5% of insulin-positive ductal cells [27]. A similar percentage has been reported by Reers

et al. that studied pancreatic tissues from 20 donors aged from 7 to 66 years [57]. The rate of islet neogenesis does not seem to be affected by aging. Importantly, Butler et al. observed that ductal cells positive for insulin were increased by 2- and 3-fold during obesity and pregnancy, respectively [32, 33]. Therefore it appears that in humans islet neogenesis from ductal cells is stimulated during metabolic situations that require an increase in insulin level. It should be noted that during both obesity and pregnancy the adaptive increase of β -cell mass is much more limited in humans compared to rodents. In pathophysiological condition such as chronic pancreatitis, Phillips et al. also observed a significant increase of insulin-positive ducts in adult human pancreas of 11 patients compared to control group [58]. In another study, the question was addressed whether β -cell neogenesis occurs in the transplanted pancreas of type 1 diabetic patients who had received a simultaneous pancreas-kidney transplant (SPK) [59]. Pancreatic tissues from 9 SPK patients and 16 nondiabetic organ donors were examined by immunohistochemistry. Remarkably, high numbers 33 to 90% of ductal cells were found to be insulin positive, and 17% to 95% of the ducts harboured insulin-positive cells in SPK patients with recurrent autoimmunity and diabetes. A high degree of neogenesis was observed in patients with the most severe β -cell loss, whereas a low degree of neogenesis was observed in normoglycaemic patients, suggesting that in the particular context pancreas transplantation, hyperglycemia and chronic inflammation may strongly stimulate β -cell neogenesis from ductal cells. Intriguingly, a study by Bouwens et al. analyzing pancreatic tissues from nine adult donors revealed that 15% of all β -cells were located in units with a diameter less than 20 μ m and without associated glucagon-, somatostatin-, or pancreatic polypeptide cells [29]. These single β -cell units were situated in or along ductules, from which they appear to bud as previously noticed in fetal and neonatal pancreas. Furthermore, simultaneous presence of Ki67-positive ductal cells (0.05%) and absence of Ki67-immunoreactive budding β -cells suggested that β -cell neogenesis depends on ductal cell proliferation and differentiation in humans.

About ten years ago murine ductal cells cultured *in vitro* were reported to form 3D clusters that differentiate to functional islet cells, which are able to respond to a glucose challenge and to reverse diabetes in mice [60]. An interesting strategy for the prospective isolation of putative progenitors from an enriched ductal cell population is also being pursued by Taniguchi and colleagues [61, 62]. The approach combines immunohistochemical analysis of mouse pancreas to define new phenotypic markers and flow cytometry cell sorting to isolate clonal cell populations that are able to differentiate toward the endocrine lineage *in vitro* or *in vivo*. The data suggest that a population of progenitor cells was present among CD133-positive ductal cells.

Koblas et al. confirmed that a subpopulation of CD133-positive cells in human islet-depleted tissue was able to differentiate into functional insulin-producing cells *in vitro* and to secrete insulin in a glucose-dependent manner [63]. Furthermore, Bonner-Weir and collaborators showed that human primary ductal cells could be isolated from islet-depleted pancreatic tissue, expanded in culture, and

triggered to differentiate towards glucose responsive islet-like clusters [64]. These results were confirmed by Gao et al. who further characterized the nature of these pancreatic progenitor cells [65]. During monolayer expansion, two subpopulations of proliferating cells were observed, CK19-positive ductal cells at an early time point (day 3) and nestin-positive cells at a later time point (day 7). Under serum-free conditions and Matrigel covering of the cells, the CK19-positive cells, but not the Nestin-positive cells, were able to form islet-like clusters that contain insulin- and glucagon-positive cells. When transplanted under the kidney capsule of nude mice, one out of five grafts demonstrated further growth with foci of both endocrine and exocrine cells. Next, Bonner-Weir and colleagues used magnetic cell sorting and antibodies raised against the ductal surface marker CA19-9 to isolate ductal cells from islet-depleted tissue [66]. Transplantation experiments of purified ductal cells versus unpurified preparations (56% CK19-positive cells only) into normoglycemic NOD/SCID mice revealed that differentiation of ductal cells to insulin-producing cells was dependent on the presence of nonductal cells, probably pancreatic stromal cells as suggested by the authors. Of interest, *in vitro* islet-to-duct plasticity has also been reported for human cells [67, 68].

Although some lineage tracing studies in rodents have provided contradictory results, most *in vivo* and *in vitro* data from both human studies indicate that cells from the ductal compartment are an attractive putative cell source for β -cell replacement strategies.

2.4. Acinar Cells. Besides the ductal origin hypothesis, a number of studies show that acinar cells might also display a certain degree of plasticity.

Melton and colleagues suggested that acinar cells and endocrine cells share a common progenitor after the ductal cell lineage has already separated [54]. On the other hand, Bouwens and colleagues proposed a variant of the ductal origin hypothesis, in which acinar cells would be able to transdifferentiate towards β -cells, through an intermediate dedifferentiated ductal-like stage [49, 69]. This model was supported by more reports. β -cell mass regeneration has been studied after streptozotocin treatment in a transgenic mouse model expressing interferon gamma under the control of the insulin promoter [70]. New β -cells appeared to result primarily from the formation of new islets from small pancreatic ducts. However, interestingly, some putative transitional cells were identified harboring both exocrine and endocrine granules, indicative of acinar cells as possible precursor cells. Similar observations were made in a parallel study after PDL [71]. Furthermore, in order to visualize better possible intermediate stages during acinar-to-ductal transdifferentiation in a PDL injury model, Lardon et al. took advantage of the fact that dexamethasone treatment inhibits the loss of amylase from acinar cells [72]. Putative transitional cells coexpressing acinus-specific (amylase) and duct-specific (CK20) markers were identified *in vivo*. Furthermore, acinus-to-islet conversion was confirmed *in vitro* after isolation of acini and identification of putative transitional cells coexpressing acinus-specific (amylase) and

β -cell-specific (insulin) markers. Several groups recently performed some *in vivo* lineage tracing analyses using acinus-specific promoters (amylase and elastase). Replication of preexisting acinar cells is seen as the major mechanism for regeneration of the acinar tissue. Moreover, acinus-to-duct transdifferentiation has been reported to occur *in vivo*, although at a very low frequency, in mouse models for pancreatitis [73], and in a mouse model that develops insulin-positive cell-containing hyperplastic ducts in response to the growth factor TGF α [74]. However, the same authors also showed that the insulin positive cells adjacent to acinus-derived ductal cells arose from preexisting insulin-positive cells and not from acinar cells. Along the same line, Stoffers and collaborator failed to observe any acinus-to- β -cell transdifferentiation in adult mice under normal conditions and after 70% pancreatectomy, PDL, or caerulein-induced pancreatitis [75]. On the other hand, Melton et al. reported that adenovirus-mediated coexpression of Pdx1, Ngn3, and MafA *in vivo* in pancreas from adult mice was sufficient to induce the transdifferentiation of mature exocrine cells into β -cells that are indistinguishable from endogenous islet β -cells in size, shape, and ultrastructure [76]. However the question remains open whether acinar cells or other possible precursor cells were reprogrammed in these experiments.

In vitro, rat exocrine cells treated with dexamethasone can convert to hepatocyte-like cells. In contrast, when cultured in low serum medium (1%) in the presence of EGF and LIF, rat exocrine cells can transdifferentiate to functional β -cells [77]. In that particular study, 10% of dedifferentiated acinar cells expressed insulin, with a total insulin content of 40 to 90% of primary β -cells, and transplantation of about 100,000 of these insulin-positive cells was sufficient to revert hyperglycemia in a diabetic nude mouse model. In an independent study, Minami et al. showed that suspension culture in presence of EGF and nicotinamide converted 5% of adult murine acinar cells to glucose responsive insulin-producing cells [78]. An adenovirus mediated *in vitro* lineage tracing study using the acinus-specific amylase or elastase promoter confirmed the identity of the starting population. In addition, acinus-to-duct transdifferentiation was shown to occur, in response to EGF-receptor signalling, through an intermediate nestin-positive stage in an *in vitro* culture of pancreatic explants [79].

Regarding human cells, there are no data available about a possible acinus-to-islet cell plasticity. One possible explanation is the selective death by apoptosis of human acinar cells when cultured *in vitro*. In contrast, human ductal cells can survive, adhere to plastic culture dish, and proliferate [80]. Nevertheless the possible acinus-to-duct transdifferentiation suggested by others [81] cannot be ruled out in this study.

2.5. Liver Cells. Liver and pancreas have a common embryological origin, both arising from the primitive gut. Therefore liver cells have been hypothesized to constitute a potential cell source for β -cell generation [82].

Ferber and collaborators demonstrated that an adenovirus-mediated expression of the key pancreatic transcription factor Pdx1 in mouse liver (intravenous infusion) resulted in transdifferentiation of hepatocytes to β -cell-like cells [83]. Pdx1 expression found in 60% of hepatocytes resulted in a 3-fold increase of plasma insulin levels, 59% as insulin, and 41% as proinsulin. These data indicate that proinsulin was processed which was substantiated by expression of the prohormone convertase 1/3. The amount of insulin produced was sufficient to reduce hyperglycemia in a diabetic mouse model. A similar approach was successfully reported with adenovirus-mediated expression of Pdx1 or Ngn3 [84] and the coexpression of NeuroD1/Beta2 and betacellulin [85] in the liver. Of note, although the overexpression of Pdx1 was able to revert the elevated blood glucose of diabetic mice, the animals died from liver inflammation most likely due to the exocrine-differentiating activity of Pdx1. Interestingly Yechoor et al. showed that gene transfer of a pancreatic key transcription factor (Ngn3 in this study) in liver leads to long-term diabetes reversal in mice [86]. However the authors demonstrated that, although insulin expression was transiently induced in terminally differentiated hepatocytes, the long-term diabetes reversal obtained in these mice was resulting from the differentiation of hepatic progenitors able to generate islet-like clusters. The phenomenon was described as “transdetermination,” that is, lineage switching in lineage-determined, but not terminally differentiated, cells.

Human adult liver cells were shown to expand *in vitro* and to transdifferentiate towards an endocrine pancreatic lineage after Pdx1 overexpression [87]. Pdx1-expressing human liver cells were found to express insulin that is stored in secretory granules, which are released in a glucose-regulated manner. When transplanted under the kidney capsule of diabetic immunodeficient mice, these cells ameliorated hyperglycemia for prolonged periods of time. Similar studies using human fetal progenitor liver cells were reported [88, 89]. Since harvesting and propagating significant numbers of primary hepatocytes from patients with diabetes would be theoretically feasible, the liver can be considered as an interesting extrapancreatic source for β -cell replacement therapy.

3. Generation of β -Cells from Adult Mesenchymal Stem Cells?

Mesenchymal stem cells (MSC) were originally identified in bone marrow by Friedenstein in 1976 as a rare, heterogeneous, non hematopoietic, and multipotent stromal population able to differentiate to mesenchymal lineages including bone, fat, and cartilage. MSC are virtually present in all organs, including the pancreas and the islets of Langerhans. It is now generally accepted that the perivascular area harbors the MSC, explaining the ubiquitous distribution of these cells in the body [90]. Interestingly, MSC can be obtained from live donors (and potentially from the patient itself) and are easily expandable *in vitro*. Therefore, despite the fact that their identity and their exact role *in vivo* have

TABLE 2: Potential sources of de novo β -cells among differentiated adult MSC: BM: bone marrow, AT: adipose tissue; UCB: umbilical cord blood.

	Rodent		Human	
	<i>in vitro</i>	<i>in vivo</i>	<i>in vitro</i>	<i>in vivo</i>
<i>Pancreatic</i>				
Islet-(derived) MSC	?	?	+	?
Exocrine-(derived) MSC	?	?	-/+	?
<i>Extra-pancreatic</i>				
BM-MSC				
AT-MSC	+	-/+	-/+	-
UCB-MSC				

not been clearly defined yet, mesenchymal stem cells have been envisaged for a broad range of therapeutic applications including type 1 diabetes [91, 92]. The data of this part are summarized in Table 2.

3.1. Islet(-Derived) MSC. We and others reported on the expansion of MSC-like cell population from isolated human islets: human islet-derived precursor cells (IPC) [93–95], NIP/Nestin-positive Islet-derived Progenitors [96], PIDM/Pancreatic Islet-Derived Mesenchymal cells [97], PHID/Proliferating Human Islet-Derived cells [98], and more [99, 100]. These cells are able to proliferate *ex vivo* and can be passaged. Interestingly, most of the groups reported a common characteristic, which is aggregation into clusters ranging in sizes between 50 and 200 μm (similar range as primary islets) under serum starvation and a subsequent increase in expression of endocrine markers. However others failed to reproduce these data of partial differentiation [101, 102]. In any case, the endocrine markers remained at very low level when compared to freshly isolated human islets. Davani et al. reported a further capacity of IPC to differentiate into functional β -cells that secrete human C-peptide in response to glucose after reimplantation of 4-day-old aggregates in mice [94].

The origin of these cells remains elusive and very controversial. Initially Gershengorn and colleagues proposed an Epithelial-to-Mesenchymal Transition (EMT) to occur from human β -cells [93]. However this idea was rapidly refuted by the same group and others after lineage tracing experiments performed in transgenic mouse models that failed to show any EMT of murine β -cells [103–106]. The overall conclusion of these four studies was that the proliferative cell population derived from cultured murine islets was not originating from β -cells, since no β -cell specific markers were identified in these cells. Efrat and collaborators recently developed a lineage tracing system similar to the techniques applied in transgenic mouse models, but now applied to human cells *in vitro* using lentiviral vectors [107]. The dual viral system relies on the β -cell specific expression of the CRE recombinase in one vector and a CMV-GFP reporter vector in another vector in which GFP expression is restricted by a “floxed” intermediate sequence. As lentiviral

vectors integrate into the genome of the transduced cells, the reporter gene will remain expressed in all cells originating from the initial pool of labeled cells. In their follow-up study, Russ et al. slightly modified the system by using a tamoxifen-inducible CRE/ER recombinase, restricting the labeling period to the duration of a short (overnight) tamoxifen pulse [108]. Human β -cells were efficiently (50%) and specifically labeled by the dual lentiviral system. This powerful technique provided evidence that human β -cells can dedifferentiate to an MSC-like cell population and proliferate when cultured *ex vivo*, in contrast to mouse β -cells, as revealed earlier from the transgenic mice studies [103–106]. Intriguingly, 40% of cells exhibiting MSC markers in culture (likely to be the cell population previously named IPC, NIP, PIDM, or PHID by others) resulted from an EMT of β -cells. On the other hand, several groups suggested, but never unequivocally demonstrated, the presence of a mesenchymal stem cell population within human islets [94, 96, 97]. In a recent study, we investigated the presence of MSC(-like cells) in freshly isolated human islets, and we identified a double-positive CD90/CD105 population representing approximately 2% of the total islet cell population. The presence of these cells inside freshly isolated human islets was confirmed by confocal microscopy [95]. An independent study validated the presence of pancreatic MSC in the periacinar, perivascular, and periductal space of human pancreas [109]. The functional significance of the presence of these cells in the islets and the possible interplay between islet-MSC, endocrine cells, and the vascular system in human islets remain to be further clarified. Altogether these data suggest that the MSC-like cell population derived from human islets in culture results from subpopulations of at least two origins: proliferation of islet-MSC and proliferation of dedifferentiated β -cells. More studies, in particular detailed lineage-tracing experiments will be needed to confirm this model.

At the moment it remains unclear whether all mesenchymal stem(-like) cells are equal and whether MSC originating from islets would be more prone to differentiate toward the endocrine lineage. Two groups determined the gene expression profiles in human islet-derived MSC [110, 111]. Both studies confirmed the common mesenchymal character of the population with the archetypical bone marrow-MSC. Remarkably cultured islet-derived IPCs are different from bone marrow-MSC (BM-MSC) in that they express a set of islet-specific genes, although at low level. Upon differentiation, following the rather basic differentiation protocols developed so far for these cells, gene expression data showed that IPCs are able to go further along the endocrine pathways than BM-MSC.

In summary, islet(-derived) MSC could be of a valuable therapeutic significance since they appear to retain some genetic characteristic making them closer to endocrine cells than other sources of MSC such as bone-marrow-derived MSC. Further studies will be needed to verify this hypothesis. Furthermore, the observation that human β -cells can dedifferentiate *ex vivo* into a mesenchymal phenotype in contrast to murine β -cells illustrates again the discrepancy in plasticity of these cells between rodents and humans.

3.2. Exocrine/Islet Depleted Tissue(-Derived) MSC. Similarly to human islet cells cultured *ex vivo*, two independent studies reported that a population of MSC(-like) cells could be derived from human pancreatic exocrine tissue. Under defined conditions, these cells could show some sign of differentiation toward the endocrine lineage [112, 113].

The exact origin of these cells remains also unclear. On the one hand, epithelial-to-mesenchymal transition from exocrine cells has been proposed by Seeberger et al. [114]. Along the same line, Fanjul et al. described coexpression of ductal markers and mesenchymal markers both in cultured human ductal-like cells *in vitro* and in ductal cells in one pancreas from a nondiabetic subject and three pancreata from patients with type 2 diabetes [115]. Shin et al. demonstrated that the transdifferentiation capacity of human ductal cells was reduced after EMT [116]. On the other hand, Sordi et al. suggested that the mesenchymal stem cell population growing out of cultured pancreatic tissue (both endocrine and exocrine fractions were tested) would be mostly originating from proliferating “pancreatic MSC” that are partly derived from the bone marrow [109]. Total bone marrow transplantations from donor GFP transgenic mice in lethally irradiated recipient mice were performed. Surprisingly, after 12 weeks, GFP-labeled bone-marrow-derived cells were found to be localized preferentially in two organs, pancreas ($4.82 \pm 4\%$ of total) and lung ($4.43 \pm 2.3\%$ of total). $18.5 \pm 4\%$ of MSC derived in culture from these pancreata were found to be GFP positive.

However once again, in absence of data on pancreatic tissues after bone marrow transplantation in humans, we can wonder how these results would be translatable to the human situation. In a study of a pancreas allograft removed 8 months after transplantation, it was found that part of pancreatic MSC expressed recipient HLA, suggesting an extrapancreatic origin of these cells [109].

Besides their putative differentiation capacity, MSC display very interesting additional characteristics. Sordi et al. reported that pancreatic MSC extracted from both endocrine and exocrine tissue and cotransplanted in mice with a minimal pancreatic islet mass facilitated the restoration of normoglycemia and neovascularization of the islet graft [109].

In conclusion, MSC can be derived from human exocrine tissue but show limited capacity of differentiation towards the endocrine pathway. Similarly to islet(-derived) MSC, the origin and role of these cells are unclear.

3.3. Extrapancreatic Sources of MSC. Bone marrow, adipose tissue, and umbilical cord blood are the main sources of BM-MSC reported so far. Hess et al. reported that transplantation of murine bone marrow cells in streptozotocin-treated mice was able to reduce hyperglycemia by initiating endogenous pancreatic regeneration. A majority of bone-marrow-derived cells were found to be localized near ductal and islet structures. Quantitative analysis of the pancreas revealed a very low frequency of donor insulin-positive cells. However the presence of donor cells was accompanied by a rapid proliferation of recipient pancreatic β -cells and by neogenesis of insulin-positive cells of recipient origin within

a week after transplantation. The mechanism behind this regeneration process remains to be clarified. The authors suggested that bone-marrow-derived endothelial cells could be involved by secreting factors that enhance tissue repair [117]. This model was confirmed by others [118]. Along the same line, Lechner et al. observed no evidence for significant transdifferentiation of labeled (GFP transgenic mice) murine bone marrow into pancreatic β -cells *in vivo* [119]. By using a mouse model for impaired bone-marrow-derived cell mobilization (Nos3 $-/-$ mice), Hasegawa et al. demonstrated that homing of donor bone-marrow-derived cells in recipient bone marrow and subsequent mobilization into the injured periphery were required for β -cell regeneration. Interestingly, simple bone marrow cell infusion without preirradiation had no effects, suggesting that injury signals are involved in triggering this process [120]. Finally, in similar transplantation experiments, Urban et al. revealed that murine-bone-marrow derived mesenchymal stem cells cooperate with bone marrow cells since neither bone marrow cells nor MSC transplantation was effective alone [121]. In contrast to all these studies and using a lineage tracing system (Ins/CRE-LoxP/EGFP), Ianus et al. claimed that transplanted murine bone marrow cells were able to differentiate themselves into functional β -cells [122]. Four to six weeks after transplantation from male mice into lethally irradiated recipient female mice, recipient mice contained Y chromosome and EGFP double-positive cells in their pancreatic islets. Of note, β -cell specificity was verified since neither bone marrow cells nor circulating peripheral blood nucleated cells of donor or recipient mice had any detectable EGFP. Cell fusion was also ruled out in this experiment.

Regarding human cells, Prockop and colleagues evaluated the competence of bone marrow MSC in a similar type of experiment. Human bone marrow MSC were delivered via intracardiac infusions in diabetic NOD/SCID mice. Although rare β -cells were found to be of human origin (i.e., BM-MSC derived), blood glucose levels were found to be decreased after some weeks. The authors suggested that human BM-MSC were able to home to and promote repair of pancreatic islets and renal glomeruli in a diabetic mouse model [123]. In a parallel study, Sordi et al. showed that human BM-MSC express a restricted set of functionally active chemokine receptors (CXCR4, CX3CR1, CXCR6, CCR1, CCR7) capable of promoting migration to pancreatic islets [124]. Butler and collaborators studied 31 human pancreases obtained at autopsy from patients who had received a bone marrow-derived graft (26 cases), a peripheral blood-derived graft (4 cases), or a combination of both peripheral blood- and bone marrow-derived stem cells (1 case) [125]. More than 4000 islets were examined in this relatively large cohort, and no pancreatic β -cells were found to be derived from donor cells (including two cases of patients with type 2 diabetes). Therefore, it appears that in humans the bone marrow compartment does not contribute to pancreatic β -cell mass maintenance in healthy individuals. Nevertheless promising clinical results on glucose metabolism were recently reported. A clinical trial in 11 patients with type 1 diabetes was designed to

test the safety and efficacy of intraportal coinfusion of insulin-producing adipose tissue-derived MSC and bone marrow cells. Differentiation of adipose tissue-derived MSC was initiated *in vitro* by a 3-day culture period in a defined medium described earlier [126]. A mean follow-up of about two years showed significant improvements of all clinical parameters related to diabetes (a decrease in insulin requirements, an increase in C-peptide levels, and absence of diabetic ketoacidosis) [127]. In a clinical trial involving 25 patients with type 2 diabetes, Ricordi and collaborators observed reduced insulin requirements and significant improvements of all metabolic variables (12-month follow-up) after an intrapancreatic infusion of autologous bone marrow cells [128]. However no data are available about the possible differentiation of MSC after implantation, and it is more likely that improved glucose metabolism is related to paracrine effects of MSC in this last study.

In vitro, several groups reported the successful derivation of functional β -cells from rodent bone marrow cells in the presence or absence of serum [129] and with addition of growth factors like nicotinamide and β -mercaptoethanol [130] and conophylline and betacellulin-delta4 [131]. After transplantation these cells can (partly) revert hyperglycemia in diabetic mice. Also murine adipose tissue-derived MSC could be efficiently converted reaching up to 48% of cells that expressed c-peptide, following a 3-stage and 10-day differentiation protocol involving activin A, sodium butyrate, β -mercaptoethanol, taurine, GLP-1, nicotinamide, and nonessential amino acids [132].

The approach is slightly different in human cells, involving the combination of defined medium and virus-mediated ectopic expression of proendocrine transcription factors. Karnieli et al. reported that overexpression of Pdx1 in BM-MSC from 9 of 14 donors can trigger their differentiation to a β -cell-like phenotype displaying about 1% of the regular insulin content and able to control the insulin release in a glucose-dependent manner *in vitro* [133]. The cells lacked expression of Beta2/NeuroD1. However transplantation into streptozotocin-treated mice resulted in further differentiation, including induction of Beta2/NeuroD1 and reduction of hyperglycemia. Similar results were obtained in a parallel study [134]. Human MSC from other sources than bone marrow were also evaluated. MSC isolated from the Wharton's jelly of the umbilical cord were differentiated to islet-like cell clusters through stepwise culturing in neuron-conditioned medium [135]. The clusters were found to express islet-specific genes and to be glucose responsive *in vitro*. Functionality was further verified after transplantation into the liver of streptozotocin-induced diabetic rats via laparotomy. The presence of characteristic secretory granules was observed by electron microscopy 12 weeks after transplantation.

In summary, the capacity of extrapancreatic mesenchymal stem cells to differentiate to β -cells *in vivo* appears to be very limited. Nevertheless, several groups reported a successful differentiation to functional β -cell-like cells *in vitro* especially from human MSC. MSC appear to display unique migratory and secretory properties (growth factors,

cytokines) that make them attractive as "helper" cells for tissue repair (improve engraftment, viability, function) (for review see [92, 136]).

4. Mechanisms for Maintenance of the Cellular Identity

A crucial aspect common to all putative cell sources will be to uncover the mechanisms that preserve and control cell identity in order to enable successful manipulation of adult cell plasticity in clinical settings. Up to now, most efforts to influence the cell identity were focused on direct gene expression (overexpression/downregulation of key transcription factors) and growth-factor-mediated activation of specific signaling pathways. A new era has started aiming at better understanding the processes that regulate gene expression. Chromatin accessibility is a determining factor blocking or facilitating expression of specific genes. Cell identity is regulated by epigenetic factors that tightly regulate the activation or repression of genes including genomic DNA methylation, histone modifications, and noncoding RNA regulation (for review please see [137, 138]). Dhawan et al. recently demonstrated that pancreatic β -cell identity is maintained by DNA methylation-mediated repression of Arx [139]. The question whether all MSC(-like cells) are equal was recently addressed. Mutskov et al. investigated the patterns of histone modifications over the insulin gene in human islets and IPC (the MSC-like population derived *ex vivo* from human islets) compared to HeLa and BM-MSC [140]. Although neither IPC nor HeLa nor BM-MSC express insulin, IPC showed significant levels of active chromatin modifications, similarly to human islets although at a more moderate level. The probable multiorigin of the IPC might obscure the interpretation of these results. However these epigenetic marks absent in the unrelated cell types (HeLa and BM-MSC) might be part of a general mechanism whereby tissue-derived precursor/stem cells are committed to a distinct specification. Non-coding RNA (such as siRNA (short interference RNA), miRNA (microRNA), and lncRNA (long non-coding RNA)) are emerging as key players in regulation of development [141]. Joglekar et al. demonstrated that the miR-30 family of miRNAs contribute to the regulation of the dedifferentiation of human fetal β -cells through epithelial-to-mesenchymal transition by negatively regulating the translation of mesenchymal genes [142].

Epigenetic reprogramming of cell types with shared developmental history could be an effective strategy for pancreatic β -cell replacement therapies. The cells may display some intrinsic commitment to become islets even during adulthood and might thus require fewer triggers to differentiate/transdifferentiate towards a β -cell lineage. Along this line, in the field of reprogramming to iPS, the notion of epigenetic "memory" inherited from the parental cell is coming forward. Bar-Nur et al. observed a preferential lineage-specific differentiation in iPS derived from human β -cells [143]. These new insights in gene regulation should help to exploit the full potential of adult cell plasticity in the perspective of cell replacement therapies to treat diseases such as type 1 diabetes.

5. Conclusions and Discussion

In conclusion, it emerges that extreme caution should be taken when translating the findings obtained from rodent studies to the human situation. Regarding *in vivo* regeneration investigated under physiological conditions or under injury, it appears that β -cell replication is the predominant mechanism occurring in mice. In humans, this process seems to be restricted to the very early postnatal life, whereas during adulthood, neogenesis from (a subpopulation of) ductal and/or acinar cells seems to be responsible for the increase of β -cell mass required in physiological situations of higher insulin demands like obesity or pregnancy. Nevertheless, the unanswered question remains which cell type could be involved: progenitor cells present in the ductal area, or fully differentiated adult cells able to transdifferentiate, or a distinct subpopulation that is able to dedifferentiate to a progenitor-like intermediate stage followed by redifferentiation to an endocrine lineage. Remarkably, both the animal model and the degree of destruction appear to be key points, as the regeneration processes can be different: β -cell mass regeneration from α cells has been observed in models of near total ablation only. Regarding *in vitro* replicative capacity of β -cells, human β -cells cannot efficiently replicate, in contrast to rodent cells. However, contrary to murine β -cells again, human β -cells can dedifferentiate to a mesenchymal-like phenotype and proliferate. These discrepancies between findings from human versus rodent studies are not as unexpected as they seem, given the major differences observed in islet cell biology field such as timing of pancreatic developmental stages [45], islet architecture and composition [144, 145], and islet innervation [146]. In the stem cell field, murine and human stem cells are notoriously dissimilar. Finally, cultured human primary cells are more prone to replicative aging than murine cells as telomere shortening limits cell replication and leads to senescence.

However, up to now, there is no way to accurately evaluate the β -cell mass in humans, neither by imaging techniques nor by physiological measurements. Studies are limited to histological analysis performed on organs at autopsy or after pancreatectomy generating static pictures that could be misinterpreted, even if careful quantification and repeated observations in different contexts can still result in strong indications. Therefore, animal models become essential offering access to a broad range of technologies obviously not applicable to humans. Among others, the recently developed genetic approaches (such as lineage tracing systems, comprising of a tissue-specific promoter and a (tamoxifen-)inducible recombination system and a reporter gene) are valuable tools to follow a dynamic process and reinforce (or sometimes challenge) earlier theories suggested by more descriptive immunohistochemistry data. Nevertheless several limitations have to be taken into consideration: a possible leakiness of the recombination system, the relative specificity of a given promoter that can display some (transient) activity in nonspecific cells, and a limited penetrance (usually less than half of the cells are actually labeled) [147, 148]. Altogether these elements might

contribute to the discrepancies between results obtained by different labs about the origin of β -cell regeneration for instance, and hopefully validation of some of the models by separate labs in different experimental contexts will clarify the situation in a near future.

Since stimulation of human β -cells replication is still elusive, other cell sources have been envisaged. Studies from the last decade revealed an unexpected aspect of plasticity from mature differentiated cells by dedifferentiation or transdifferentiation. Of interest, it appears that regeneration does not always require recapitulation of the embryological development as needed for efficient differentiation of ES cells. For instance, the recent discovery of α -to- β -cell plasticity does not appear to correlate with any developmental process [7]. Murine and human ductal cells, rodent acinar cells (human ones cannot be maintained in culture), and human liver cells could be efficiently converted to functional β -cells able to revert hyperglycemia in a diabetic mouse model. Although the potential contribution of MSC to islet regeneration in a physiological situation remains unclear and their origin and function are still elusive, islet-derived mesenchymal stem cells seem to display specific genetic and epigenetic marks that could make them more prone to differentiation towards the endocrine compartment than extrapancreatic sources of MSC. This suggests that all mesenchymal stem cells (-like cells) are not equal. In addition MSC, in particular from humans, revealed additional properties (like homing to injury site and secretion of favorable growth factors) that may be of clinical use. Therefore, further investigations will be required to determine if islet-(derived-) MSC can be stimulated to contribute in any way to β -cell mass regeneration. Another approach successfully tested has been to force expression of proendocrine transcription factors *in vivo* in the liver and in the exocrine tissue. However no lineage tracing experiments have been performed in these studies, and the origin of the newly formed β -cells needs to be identified.

Finally, there is currently no unique optimal alternative cell source for β -cell (re)generation. Therefore a crucial aspect common to all putative cell sources will be to further uncover the mechanisms that preserve and control cell identity in order to enable successful manipulation of adult cell plasticity for clinical application.

Acknowledgment

This work has been supported by the Dutch Diabetes Fonds, the Stichting DON, The Bontius Foundation and the DCTI consortium.

References

- [1] E. A. Ryan, B. W. Paty, P. A. Senior et al., "Five-year follow-up after clinical islet transplantation," *Diabetes*, vol. 54, no. 7, pp. 2060–2069, 2005.
- [2] H. de Kort, E. J. de Koning, T. J. Rabelink, J. A. Bruijn, and I. M. Bajema, "Islet transplantation in type 1 diabetes," *British Medical Journal*, vol. 342, p. d217, 2011.

- [3] A. Granger and J. A. Kushner, "Cellular origins of β -cell regeneration: a legacy view of historical controversies," *Journal of Internal Medicine*, vol. 266, no. 4, pp. 325–338, 2009.
- [4] C. Aguayo-Mazzucato and S. Bonner-Weir, "Stem cell therapy for type 1 diabetes mellitus," *Nature Reviews Endocrinology*, vol. 6, no. 3, pp. 139–148, 2010.
- [5] P. Collombat, X. Xu, H. Heimberg, and A. Mansouri, "Pancreatic beta-cells: from generation to regeneration," *Seminars in Cell and Developmental Biology*, vol. 21, no. 8, pp. 838–844, 2010.
- [6] C. H. Chung and F. Levine, "Adult pancreatic alpha cells: a new source of cells for beta-cell regeneration," *Review of Diabetic Studies*, vol. 7, no. 2, pp. 124–131, 2010.
- [7] R. Desgraz, C. Bonal, and P. L. Herrera, " β -Cell regeneration: the pancreatic intrinsic faculty," *Trends in Endocrinology and Metabolism*, vol. 22, no. 1, pp. 34–43, 2011.
- [8] S. Bonner-Weir, W. C. Li, L. Ouziel-Yahalom, L. Guo, G. C. Weir, and A. Sharma, " β -cell growth and regeneration: replication is only part of the story," *Diabetes*, vol. 59, no. 10, pp. 2340–2348, 2010.
- [9] J. Domínguez-Bendala, L. Inverardi, and C. Ricordi, "Stem cell-derived islet cells for transplantation," *Current Opinion in Organ Transplantation*, 2010.
- [10] D. Van Hoof, K. A. D'Amour, and M. S. German, "Derivation of insulin-producing cells from human embryonic stem cells," *Stem Cell Research*, vol. 3, no. 2-3, pp. 73–87, 2009.
- [11] M. C. Nostro, F. Sarangi, S. Ogawa et al., "Stage-specific signaling through TGF β family members and WNT regulates patterning and pancreatic specification of human pluripotent stem cells," *Development*, vol. 138, no. 5, pp. 861–871, 2011.
- [12] K. Takahashi and S. Yamanaka, "Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors," *Cell*, vol. 126, no. 4, pp. 663–676, 2006.
- [13] K. Tateishi, J. He, O. Taranova, G. Liang, A. C. D'Alessio, and Y. Zhang, "Generation of insulin-secreting islet-like clusters from human skin fibroblasts," *Journal of Biological Chemistry*, vol. 283, no. 46, pp. 31601–31607, 2008.
- [14] D. Zhang, W. Jiang, M. Liu et al., "Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells," *Cell Research*, vol. 19, no. 4, pp. 429–438, 2009.
- [15] Z. Alipio, W. Liao, E. J. Roemer et al., "Reversal of hyperglycemia in diabetic mouse models using induced-pluripotent stem (iPS)-derived pancreatic β -like cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 30, pp. 13426–13431, 2010.
- [16] R. Maehr, S. Chen, M. Snitow et al., "Generation of pluripotent stem cells from patients with type 1 diabetes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 37, pp. 15768–15773, 2009.
- [17] C. Mummery, "Induced pluripotent stem cells—a cautionary note," *New England Journal of Medicine*, vol. 364, no. 22, pp. 2160–2162, 2011.
- [18] Y. Dor, J. Brown, O. I. Martinez, and D. A. Melton, "Adult pancreatic β -cells are formed by self-duplication rather than stem-cell differentiation," *Nature*, vol. 429, no. 6987, pp. 41–46, 2004.
- [19] T. Nir, D. A. Melton, and Y. Dor, "Recovery from diabetes in mice by β cell regeneration," *Journal of Clinical Investigation*, vol. 117, no. 9, pp. 2553–2561, 2007.
- [20] M. Teta, S. Y. Long, L. M. Wartschow, M. M. Rankin, and J. A. Kushner, "Very slow turnover of β -cells in aged adult mice," *Diabetes*, vol. 54, no. 9, pp. 2557–2567, 2005.
- [21] S. I. Tschen, S. Dhawan, T. Gurlo, and A. Bhushan, "Age-dependent decline in β -cell proliferation restricts the capacity of β -cell regeneration in mice," *Diabetes*, vol. 58, no. 6, pp. 1312–1320, 2009.
- [22] J. Krishnamurthy, M. R. Ramsey, K. L. Ligon et al., "p16INK4a induces an age-dependent decline in islet regenerative potential," *Nature*, vol. 443, no. 7110, pp. 453–457, 2006.
- [23] K. Brennand, D. Huangfu, and D. Melton, "All beta cells contribute equally to islet growth and maintenance," *PLoS Biology*, vol. 5, no. 7, p. e163, 2007.
- [24] M. Teta, M. M. Rankin, S. Y. Long, G. M. Stein, and J. A. Kushner, "Growth and regeneration of adult β cells does not involve specialized progenitors," *Developmental Cell*, vol. 12, no. 5, pp. 817–826, 2007.
- [25] S. J. Salpeter, A. M. Klein, D. Huangfu, J. Grimsby, and Y. Dor, "Glucose and aging control the quiescence period that follows pancreatic beta cell replication," *Development*, vol. 137, no. 19, pp. 3205–3213, 2010.
- [26] S. Porat, N. Weinberg-Corem, S. Tornovsky-Babaey et al., "Control of pancreatic β cell regeneration by glucose metabolism," *Cell Metabolism*, vol. 13, no. 4, pp. 440–449, 2011.
- [27] J. J. Meier, A. E. Butler, Y. Saisho et al., " β -cell replication is the primary mechanism subserving the postnatal expansion of β -cell mass in humans," *Diabetes*, vol. 57, no. 6, pp. 1584–1594, 2008.
- [28] S. A. Kassem, I. Ariel, P. S. Thornton, I. Scheimberg, and B. Glaser, " β -Cell proliferation and apoptosis in the developing normal human pancreas and in hyperinsulinism of infancy," *Diabetes*, vol. 49, no. 8, pp. 1325–1333, 2000.
- [29] L. Bouwens and D. G. Pipeleers, "Extra-insular beta cells associated with ductules are frequent in adult human pancreas," *Diabetologia*, vol. 41, no. 6, pp. 629–633, 1998.
- [30] M. Cnop, S. J. Hughes, M. Igoillo-Esteve et al., "The long lifespan and low turnover of human islet beta cells estimated by mathematical modeling of lipofuscin accumulation," *Diabetologia*, vol. 53, no. 2, pp. 321–330, 2010.
- [31] S. Y. Perl, J. A. Kushner, B. A. Buchholz et al., "Significant human β -cell turnover is limited to the first three decades of life as determined by in vivo thymidine analog incorporation and radiocarbon dating," *Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 10, pp. E234–E239, 2010.
- [32] A. E. Butler, J. Janson, S. Bonner-Weir, R. Ritzel, R. A. Rizza, and P. C. Butler, " β -cell deficit and increased β -cell apoptosis in humans with type 2 diabetes," *Diabetes*, vol. 52, no. 1, pp. 102–110, 2003.
- [33] A. E. Butler, L. Cao-Minh, R. Galasso et al., "Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy," *Diabetologia*, vol. 53, no. 10, pp. 2167–2176, 2010.
- [34] S. Rieck and K. H. Kaestner, "Expansion of β -cell mass in response to pregnancy," *Trends in Endocrinology and Metabolism*, vol. 21, no. 3, pp. 151–158, 2010.
- [35] B. A. Menge, A. Tannapfel, O. Belyaev et al., "Partial pancreatectomy in adult humans does not provoke β -cell regeneration," *Diabetes*, vol. 57, no. 1, pp. 142–149, 2008.
- [36] A. Hayek, G. M. Beattie, V. Cirulli, A. D. Lopez, C. Ricordi, and J. S. Rubin, "Growth factor/matrix-induced proliferation

- of human adult β - cells," *Diabetes*, vol. 44, no. 12, pp. 1458–1460, 1995.
- [37] G. M. Beattie, V. Cirulli, A. D. Lopez, and A. Hayek, "Ex Vivo Expansion of Human Pancreatic Endocrine Cells," *Journal of Clinical Endocrinology and Metabolism*, vol. 82, no. 6, pp. 1852–1856, 1997.
- [38] V. H. Lefebvre, T. Otonkoski, J. Ustinov, M. A. Huotari, D. G. Pipeleers, and L. Bouwens, "Culture of adult human islet preparations with hepatocyte growth factor and 804G matrix is mitogenic for duct cells but not for β -cells: effect of HGF on human 2-cells," *Diabetes*, vol. 47, no. 1, pp. 134–137, 1998.
- [39] G. Parnaud, D. Bosco, T. Berney et al., "Proliferation of sorted human and rat beta cells," *Diabetologia*, vol. 51, no. 1, pp. 91–100, 2008.
- [40] P. Collombat, X. Xu, P. Ravassard et al., "The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into α and subsequently β cells," *Cell*, vol. 138, no. 3, pp. 449–462, 2009.
- [41] F. Thorel, V. Népote, I. Avril et al., "Conversion of adult pancreatic α -cells to β -cells after extreme β -cell loss," *Nature*, vol. 464, no. 7292, pp. 1149–1154, 2010.
- [42] C. H. Chung, E. Hao, R. Piran, E. Keinan, and F. Levine, "Pancreatic β -cell neogenesis by direct conversion from mature α -cells," *Stem Cells*, vol. 28, no. 9, pp. 1630–1638, 2010.
- [43] J. Gromada, I. Franklin, and C. B. Wollheim, " α -cells of the endocrine pancreas: 35 years of research but the enigma remains," *Endocrine Reviews*, vol. 28, no. 1, pp. 84–116, 2007.
- [44] P. L. Herrera, "Adult insulin- and glucagon-producing cells differentiate from two independent cell lineages," *Development*, vol. 127, no. 11, pp. 2317–2322, 2000.
- [45] K. Piper, S. Brickwood, L. W. Turnpenny et al., "Beta cell differentiation during early human pancreas development," *Journal of Endocrinology*, vol. 181, no. 1, pp. 11–23, 2004.
- [46] L. Rosenberg, W. P. Duguid, and A. I. Vinik, "The effect of cellophane wrapping of the pancreas in the Syrian golden hamster: autoradiographic observations," *Pancreas*, vol. 4, no. 1, pp. 31–37, 1989.
- [47] S. Bonner-Weir, L. A. Baxter, G. T. Schupp, and F. E. Smith, "A second pathway for regeneration of adult exocrine and endocrine pancreas: a possible recapitulation of embryonic development," *Diabetes*, vol. 42, no. 12, pp. 1715–1720, 1993.
- [48] D. Gu and N. Sarvetnick, "Epithelial cell proliferation and islet neogenesis in IFN- γ transgenic mice," *Development*, vol. 118, no. 1, pp. 33–46, 1993.
- [49] R. N. Wang, G. Klöppel, and L. Bouwens, "Duct- to islet-cell differentiation and islet growth in the pancreas of duct-ligated adult rats," *Diabetologia*, vol. 38, no. 12, pp. 1405–1411, 1995.
- [50] A. Sharma, D. H. Zangen, P. Reitz et al., "The homeodomain protein IDX-1 increases after an early burst of proliferation during pancreatic regeneration," *Diabetes*, vol. 48, no. 3, pp. 507–513, 1999.
- [51] A. Inada, C. Nienaber, H. Katsuta et al., "Carbonic anhydrase II-positive pancreatic cells are progenitors for both endocrine and exocrine pancreas after birth," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 50, pp. 19915–19919, 2008.
- [52] X. Xu, J. D'Hoker, G. Stangé et al., " β cells can be generated from endogenous progenitors in injured adult mouse pancreas," *Cell*, vol. 132, no. 2, pp. 197–207, 2008.
- [53] M. Solar, C. Cardalda, I. Houbracken et al., "Pancreatic exocrine duct cells give rise to insulin-producing β cells during embryogenesis but not after birth," *Developmental Cell*, vol. 17, no. 6, pp. 849–860, 2009.
- [54] G. Gu, J. Dubauskaite, and D. A. Melton, "Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors," *Development*, vol. 129, no. 10, pp. 2447–2457, 2002.
- [55] D. Kopinke and L. C. Murtaugh, "Exocrine-to-endocrine differentiation is detectable only prior to birth in the uninjured mouse pancreas," *BMC Developmental Biology*, vol. 10, 2010.
- [56] J. L. Kopp, C. L. Dubois, A. E. Schaffer et al., "Sox9+ ductal cells are multipotent progenitors throughout development but do not produce new endocrine cells in the normal or injured adult pancreas," *Development*, vol. 138, no. 4, pp. 653–665, 2011.
- [57] C. Reers, S. Erbel, I. Esposito et al., "Impaired islet turnover in human donor pancreata with aging," *European Journal of Endocrinology*, vol. 160, no. 2, pp. 185–191, 2009.
- [58] J. M. Phillips, L. O'Reilly, C. Bland, A. K. Foulis, and A. Cooke, "Patients with chronic pancreatitis have islet progenitor cells in their ducts, but reversal of overt diabetes in NOD mice by anti-CD3 shows no evidence for islet regeneration," *Diabetes*, vol. 56, no. 3, pp. 634–640, 2007.
- [59] A. Martin-Pagola, G. Sisino, G. Allende et al., "Insulin protein and proliferation in ductal cells in the transplanted pancreas of patients with type 1 diabetes and recurrence of autoimmunity," *Diabetologia*, vol. 51, no. 10, pp. 1803–1813, 2008.
- [60] V. K. Ramiya, M. Maraist, K. E. Arfors, D. A. Schatz, A. B. Peck, and J. G. Cornelius, "Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells," *Nature Medicine*, vol. 6, no. 3, pp. 278–282, 2000.
- [61] A. Suzuki, H. Nakauchi, and H. Taniguchi, "Prospective isolation of multipotent pancreatic progenitors using flow-cytometric cell sorting," *Diabetes*, vol. 53, no. 8, pp. 2143–2152, 2004.
- [62] Y. Oshima, A. Suzuki, K. Kawashimo, M. Ishikawa, N. Ohkohchi, and H. Taniguchi, "Isolation of mouse pancreatic ductal progenitor cells expressing CD133 and c-Met by flow cytometric cell sorting," *Gastroenterology*, vol. 132, no. 2, pp. 720–732, 2007.
- [63] T. Koblas, L. Pektorova, K. Zacharovova et al., "Differentiation of CD133-positive pancreatic cells into insulin-producing islet-like cell clusters," *Transplantation Proceedings*, vol. 40, no. 2, pp. 415–418, 2008.
- [64] S. Bonner-Weir, M. Taneja, G. C. Weir et al., "In vitro cultivation of human islets from expanded ductal tissue," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 14, pp. 7999–8004, 2000.
- [65] R. Gao, J. Ustinov, M. A. Pulkkinen, K. Lundin, O. Korsgren, and T. Otonkoski, "Characterization of endocrine progenitor cells and critical factors for their differentiation in human adult pancreatic cell culture," *Diabetes*, vol. 52, no. 8, pp. 2007–2015, 2003.
- [66] S. Yatoh, R. Dodge, T. Akashi et al., "Differentiation of affinity-purified human pancreatic duct cells to β -cells," *Diabetes*, vol. 56, no. 7, pp. 1802–1809, 2007.
- [67] J. Kerr-Conte, F. Pattou, M. Lecomte-Houcke et al., "Ductal cyst formation in collagen-embedded adult human islet preparations: a means to the reproduction of nesidioblastosis in vitro," *Diabetes*, vol. 45, no. 8, pp. 1108–1114, 1996.

- [68] A. M. Jamal, M. Lipsett, R. Sladek, S. Laganière, S. Hanley, and L. Rosenberg, "Morphogenetic plasticity of adult human pancreatic islets of Langerhans," *Cell Death and Differentiation*, vol. 12, no. 7, pp. 702–712, 2005.
- [69] L. Baeyens and L. Bouwens, "Can β -cells be derived from exocrine pancreas?" *Diabetes, Obesity and Metabolism*, vol. 10, supplement 4, pp. 170–178, 2008.
- [70] D. Gu, M. Arnush, and N. Sarvetnick, "Endocrine/exocrine intermediate cells in streptozotocin-treated ins- IFN- γ transgenic mice," *Pancreas*, vol. 15, no. 3, pp. 246–250, 1997.
- [71] E. Bertelli and M. Bendayan, "Intermediate endocrine-acinar pancreatic cells in duct ligation conditions," *American Journal of Physiology*, vol. 273, no. 5, pp. C1641–C1649, 1997.
- [72] J. Lardon, N. Huyens, I. Rooman, and L. Bouwens, "Exocrine cell transdifferentiation in dexamethasone-treated rat pancreas," *Virchows Archiv*, vol. 444, no. 1, pp. 61–65, 2004.
- [73] O. Strobel, Y. Dor, J. Alsina et al., "In vivo lineage tracing defines the role of acinar-to-ductal transdifferentiation in inflammatory ductal metaplasia," *Gastroenterology*, vol. 133, no. 6, pp. 1999–2009, 2007.
- [74] S. A. Blaine, K. C. Ray, R. Anunobi, M. A. Gannon, M. K. Washington, and A. L. Means, "Adult pancreatic acinar cells give rise to ducts but not endocrine cells in response to growth factor signaling," *Development*, vol. 137, no. 14, pp. 2289–2296, 2010.
- [75] B. M. Desai, J. Oliver-Krasinski, D. D. De Leon et al., "Preexisting pancreatic acinar cells contribute to acinar cell, but not islet β cell, regeneration," *Journal of Clinical Investigation*, vol. 117, no. 4, pp. 971–977, 2007.
- [76] Q. Zhou, J. Brown, A. Kanarek, J. Rajagopal, and D. A. Melton, "In vivo reprogramming of adult pancreatic exocrine cells to β -cells," *Nature*, vol. 455, no. 7213, pp. 627–632, 2008.
- [77] L. Baeyens, S. De Breuck, J. Lardon, J. K. Mfopou, I. Rooman, and L. Bouwens, "In vitro generation of insulin-producing beta cells from adult exocrine pancreatic cells," *Diabetologia*, vol. 48, no. 1, pp. 49–57, 2005.
- [78] K. Minami, M. Okuno, K. Miyawaki et al., "Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 42, pp. 15116–15121, 2005.
- [79] A. L. Means, I. M. Meszoely, K. Suzuki et al., "Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates," *Development*, vol. 132, no. 16, pp. 3767–3776, 2005.
- [80] C. N. Street, J. R. Lakey, R. V. Rajotte et al., "Enriched human pancreatic ductal cultures obtained from selective death of acinar cells express pancreatic and duodenal homeobox gene-1 age-dependently," *Review of Diabetic Studies*, vol. 1, no. 2, pp. 66–79, 2004.
- [81] P. A. Hall and N. R. Lemoine, "Rapid acinar to ductal transdifferentiation in cultured human exocrine pancreas," *Journal of Pathology*, vol. 166, no. 2, pp. 97–103, 1992.
- [82] K. S. Zaret and M. Grompe, "Generation and regeneration of cells of the liver and pancreas," *Science*, vol. 322, no. 5907, pp. 1490–1494, 2008.
- [83] S. Ferber, A. Halkin, H. Cohen et al., "Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia," *Nature Medicine*, vol. 6, no. 5, pp. 568–572, 2000.
- [84] A. Y. Wang, A. Ehrhardt, H. Xu, and M. A. Kay, "Adenovirus transduction is required for the correction of diabetes using Pdx-1 or neurogenin-3 in the liver," *Molecular Therapy*, vol. 15, no. 2, pp. 255–263, 2007.
- [85] H. Kojima, M. Fujimiya, K. Matsumura et al., "NeuroD-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice," *Nature Medicine*, vol. 9, no. 5, pp. 596–603, 2003.
- [86] V. Yechoor, V. Liu, C. Espiritu et al., "Neurogenin3 is sufficient for transdetermination of hepatic progenitor cells into neo-islets in vivo but not transdifferentiation of hepatocytes," *Developmental Cell*, vol. 16, no. 3, pp. 358–373, 2009.
- [87] T. Sapir, K. Shternhall, I. Meivar-Levy et al., "Cell-replacement therapy for diabetes: generating functional insulin-producing tissue from adult human liver cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 22, pp. 7964–7969, 2005.
- [88] M. Zalzman, S. Gupta, R. K. Giri et al., "Reversal of hyperglycemia in mice by using human expandable insulin-producing cells differentiated from fetal liver progenitor cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 12, pp. 7253–7258, 2003.
- [89] M. Zalzman, L. Anker-Kitai, and S. Efrat, "Differentiation of human liver-derived, insulin-producing cells toward the β -cell phenotype," *Diabetes*, vol. 54, no. 9, pp. 2568–2575, 2005.
- [90] M. Crisan, S. Yap, L. Casteilla et al., "A perivascular origin for mesenchymal stem cells in multiple human organs," *Cell Stem Cell*, vol. 3, no. 3, pp. 301–313, 2008.
- [91] M. Dominici, K. Le Blanc, I. Mueller et al., "Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement," *Cytotherapy*, vol. 8, no. 4, pp. 315–317, 2006.
- [92] C. W. Chen, E. Montelatici, M. Crisan et al., "Perivascular multi-lineage progenitor cells in human organs: regenerative units, cytokine sources or both?" *Cytokine and Growth Factor Reviews*, vol. 20, no. 5-6, pp. 429–434, 2009.
- [93] M. C. Gershengorn, A. A. Hardikar, C. Wei, E. Ceras-Raaka, B. Marcus-Samuels, and B. M. Raaka, "Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells," *Science*, vol. 306, no. 5705, pp. 2261–2264, 2004.
- [94] B. Davani, L. Ikonou, B. M. Raaka et al., "Human islet-derived precursor cells are mesenchymal stromal cells that differentiate and mature to hormone-expressing cells in vivo," *Stem Cells*, vol. 25, no. 12, pp. 3215–3222, 2007.
- [95] F. Carlotti, A. Zaldumbide, C. J. Loomans et al., "Isolated human islets contain a distinct population of mesenchymal stem cells," *Islets*, vol. 2, no. 3, 2010.
- [96] H. Zulewski, E. J. Abraham, M. J. Gerlach et al., "Multipotent nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes," *Diabetes*, vol. 50, no. 3, pp. 521–533, 2001.
- [97] R. Gallo, F. Gambelli, B. Gava et al., "Generation and expansion of multipotent mesenchymal progenitor cells from cultured human pancreatic islets," *Cell Death and Differentiation*, vol. 14, no. 11, pp. 1860–1871, 2007.
- [98] L. Ouziel-Yahalom, M. Zalzman, L. Anker-Kitai et al., "Expansion and redifferentiation of adult human pancreatic islet cells," *Biochemical and Biophysical Research Communications*, vol. 341, no. 2, pp. 291–298, 2006.
- [99] A. Lechner, A. L. Nolan, R. A. Blacken, and J. F. Habener, "Redifferentiation of insulin-secreting cells after in vitro

- expansion of adult human pancreatic islet tissue," *Biochemical and Biophysical Research Communications*, vol. 327, no. 2, pp. 581–588, 2005.
- [100] M. Eberhardt, P. Salmon, M.-A. von Mach et al., "Multipotential nestin and Isl-1 positive mesenchymal stem cells isolated from human pancreatic islets," *Biochemical and Biophysical Research Communications*, vol. 345, no. 3, pp. 1167–1176, 2006.
- [101] G. M. Beattie, P. Itkin-Ansari, V. Cirulli et al., "Sustained proliferation of PDX-1+ cells derived from human islets," *Diabetes*, vol. 48, no. 5, pp. 1013–1019, 1999.
- [102] A. G. Kayali, L. E. Flores, A. D. Lopez et al., "Limited capacity of human adult islets expanded in vitro to redifferentiate into insulin-producing β -cells," *Diabetes*, vol. 56, no. 3, pp. 703–708, 2007.
- [103] R. A. Morton, E. Geras-Raaka, L. M. Wilson, B. M. Raaka, and M. C. Gershengorn, "Endocrine precursor cells from mouse islets are not generated by epithelial-to-mesenchymal transition of mature beta cells," *Molecular and Cellular Endocrinology*, vol. 270, no. 1-2, pp. 87–93, 2007.
- [104] L. G. Chase, F. Ulloa-Montoya, B. L. Kidder, and C. M. Verfaillie, "Islet-derived fibroblast-like cells are not derived via epithelial-mesenchymal transition from Pdx-1 or insulin-positive cells," *Diabetes*, vol. 56, no. 1, pp. 3–7, 2007.
- [105] F. Atouf, H. P. Cheol, K. Pechhold, M. Ta, Y. Choi, and N. L. Lumelsky, "No evidence for mouse pancreatic β -cell epithelial-mesenchymal transition in vitro," *Diabetes*, vol. 56, no. 3, pp. 699–702, 2007.
- [106] N. Weinberg, L. Ouziel-Yahalom, S. Knoller, S. Efrat, and Y. Dor, "Lineage tracing evidence for in vitro dedifferentiation but rare proliferation of mouse pancreatic β -cells," *Diabetes*, vol. 56, no. 5, pp. 1299–1304, 2007.
- [107] H. A. Russ, Y. Bar, P. Ravassard, and S. Efrat, "In vitro proliferation of cells derived from adult human β -cells revealed by cell-lineage tracing," *Diabetes*, vol. 57, no. 6, pp. 1575–1583, 2008.
- [108] H. A. Russ, P. Ravassard, J. Kerr-Conte, F. Pattou, and S. Efrat, "Epithelial-mesenchymal transition in cells expanded in vitro from lineage-traced adult human pancreatic beta cells," *PLoS ONE*, vol. 4, no. 7, Article ID e6417, 2009.
- [109] V. Sordi, R. Melzi, A. Mercalli et al., "Mesenchymal cells appearing in pancreatic tissue culture are bone marrow-derived stem cells with the capacity to improve transplanted islet function," *Stem Cells*, vol. 28, no. 1, pp. 140–151, 2010.
- [110] B. Kutlu, A. G. Kayali, S. Jung et al., "Meta-analysis of gene expression in human pancreatic islets after in vitro expansion," *Physiological Genomics*, vol. 39, no. 1, pp. 72–81, 2009.
- [111] C. Limbert, R. Ebert, T. Schilling et al., "Functional signature of human islet-derived precursor cells compared to bone marrow-derived mesenchymal stem cells," *Stem Cells and Development*, vol. 19, no. 5, pp. 679–691, 2010.
- [112] K. L. Seeberger, J. M. Dufour, A. M. James Shapiro, J. R. T. Lakey, R. V. Rajotte, and G. S. Korbitt, "Expansion of mesenchymal stem cells from human pancreatic ductal epithelium," *Laboratory Investigation*, vol. 86, no. 2, pp. 141–153, 2006.
- [113] R. M. Baertschiger, D. Bosco, P. Morel et al., "Mesenchymal stem cells derived from human exocrine pancreas express transcription factors implicated in beta-cell development," *Pancreas*, vol. 37, no. 1, pp. 75–84, 2008.
- [114] K. L. Seeberger, A. Eshpeter, R. V. Rajotte, and G. S. Korbitt, "Epithelial cells within the human pancreas do not coexpress mesenchymal antigens: epithelial-mesenchymal transition is an artifact of cell culture," *Laboratory Investigation*, vol. 89, no. 2, pp. 110–121, 2009.
- [115] M. Fanjul, V. Gmyr, C. Sengenès et al., "Evidence for epithelial-mesenchymal transition in adult human pancreatic exocrine cells," *Journal of Histochemistry and Cytochemistry*, vol. 58, no. 9, pp. 807–823, 2010.
- [116] J. A. Shin, O. K. Hong, H. J. Lee et al., "Transforming growth factor- β induces epithelial to mesenchymal transition and suppresses the proliferation and transdifferentiation of cultured human pancreatic duct cells," *Journal of Cellular Biochemistry*, vol. 112, no. 1, pp. 179–188, 2011.
- [117] D. Hess, L. Li, M. Martin et al., "Bone marrow-derived stem cells initiate pancreatic regeneration," *Nature Biotechnology*, vol. 21, no. 7, pp. 763–770, 2003.
- [118] X. Gao, L. Song, K. Shen, H. Wang, W. Niu, and X. Qin, "Transplantation of bone marrow derived cells promotes pancreatic islet repair in diabetic mice," *Biochemical and Biophysical Research Communications*, vol. 371, no. 1, pp. 132–137, 2008.
- [119] A. Lechner, Y. G. Yang, R. A. Blacken, L. Wang, A. L. Nolan, and J. F. Habener, "No evidence for significant transdifferentiation of bone marrow into pancreatic β -Cells in vivo," *Diabetes*, vol. 53, no. 3, pp. 616–623, 2004.
- [120] Y. Hasegawa, T. Ogihara, T. Yamada et al., "Bone marrow (BM) transplantation promotes β -cell regeneration after acute injury through BM cell mobilization," *Endocrinology*, vol. 148, no. 5, pp. 2006–2015, 2007.
- [121] V. S. Urbán, J. Kiss, J. Kovács et al., "Mesenchymal stem cells cooperate with bone marrow cells in therapy of diabetes," *Stem Cells*, vol. 26, no. 1, pp. 244–253, 2008.
- [122] A. Ianus, G. G. Holz, N. D. Theise, and M. A. Hussain, "In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion," *Journal of Clinical Investigation*, vol. 111, no. 6, pp. 843–850, 2003.
- [123] R. H. Lee, M. J. Seo, R. L. Reger et al., "Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 46, pp. 17438–17443, 2006.
- [124] V. Sordi, M. L. Malosio, F. Marchesi et al., "Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets," *Blood*, vol. 106, no. 2, pp. 419–427, 2005.
- [125] A. E. Butler, A. Huang, P. N. Rao et al., "Hematopoietic stem cells derived from adult donors are not a source of pancreatic β -cells in adult nondiabetic humans," *Diabetes*, vol. 56, no. 7, pp. 1810–1816, 2007.
- [126] K. Timper, D. Seboek, M. Eberhardt et al., "Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells," *Biochemical and Biophysical Research Communications*, vol. 341, no. 4, pp. 1135–1140, 2006.
- [127] A. V. Vanikar, S. D. Dave, U. G. Thakkar, and H. L. Trivedi, "Cotransplantation of adipose tissue-derived insulin-secreting mesenchymal stem cells and hematopoietic stem cells: a novel therapy for insulin-dependent diabetes mellitus," *Stem Cells International*, vol. 2010, Article ID 582382, p. 5, 2010.

- [128] E. J. Estrada, F. Valacchi, E. Nicora et al., "Combined treatment of intrapancreatic autologous bone marrow stem cells and hyperbaric oxygen in type 2 diabetes mellitus," *Cell Transplantation*, vol. 17, no. 12, pp. 1295–1304, 2008.
- [129] S. H. Oh, T. M. Muzzonigro, S. H. Bae, J. M. LaPlante, H. M. Hatch, and B. E. Petersen, "Adult bone marrow-derived cells trans differentiating into insulin-producing cells for the treatment of type I diabetes," *Laboratory Investigation*, vol. 84, no. 5, pp. 607–617, 2004.
- [130] L. B. Chen, X. B. Jiang, and L. Yang, "Differentiation of rat marrow mesenchymal stem cells into pancreatic islet beta-cells," *World Journal of Gastroenterology*, vol. 10, no. 20, pp. 3016–3020, 2004.
- [131] E. Hisanaga, K. Y. Park, S. Yamada et al., "A simple method to induce differentiation of murine bone marrow mesenchymal cells to insulin-producing cells using conophylline and betacellulin-delta4," *Endocrine Journal*, vol. 55, no. 3, pp. 535–543, 2008.
- [132] V. Chandra, S. G. S. Phadnis, P. D. Nair, and R. R. Bhonde, "Generation of pancreatic hormone-expressing islet-like cell aggregates from murine adipose tissue-derived stem cells," *Stem Cells*, vol. 27, no. 8, pp. 1941–1953, 2009.
- [133] O. Karnieli, Y. Izhar-Prato, S. Bulvik, and S. Efrat, "Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation," *Stem Cells*, vol. 25, no. 11, pp. 2837–2844, 2007.
- [134] Y. Li, R. Zhang, H. Qiao et al., "Generation of insulin-producing cells from PDX-1 gene-modified human mesenchymal stem cells," *Journal of Cellular Physiology*, vol. 211, no. 1, pp. 36–44, 2007.
- [135] K. C. Chao, K. F. Chao, Y. S. Fu, and S. H. Liu, "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, Article ID e1451, 2008.
- [136] V. Sordi and L. Piemonti, "Mesenchymal stem cells as feeder cells for pancreatic islet transplants," *Review of Diabetic Studies*, vol. 7, no. 2, pp. 132–143, 2010.
- [137] M. J. Barrero, S. Boué, and J. C. Izpisua Belmonte, "Epigenetic Mechanisms that Regulate Cell Identity," *Cell Stem Cell*, vol. 7, no. 5, pp. 565–570, 2010.
- [138] M. Berdasco and M. Esteller, "Aberrant epigenetic landscape in cancer: how cellular identity goes awry," *Developmental Cell*, vol. 19, no. 5, pp. 698–711, 2010.
- [139] S. Dhawan, S. Georgia, S. -I. Tschen, G. Fan, and A. Bhushan, "Pancreatic β cell identity is maintained by DNA methylation-mediated repression of *Arx*," *Developmental Cell*, vol. 20, no. 4, pp. 419–429, 2011.
- [140] V. Mutskov, B. M. Raaka, G. Felsenfeld, and M. C. Gershengorn, "The human insulin gene displays transcriptionally active epigenetic marks in islet-derived mesenchymal precursor cells in the absence of insulin expression," *Stem Cells*, vol. 25, no. 12, pp. 3223–3233, 2007.
- [141] S. van Leeuwen and H. Mikkers, "Long non-coding RNAs: guardians of development," *Differentiation*, vol. 80, no. 4-5, pp. 175–183, 2010.
- [142] M. V. Joglekar, D. Patil, V. M. Joglekar et al., "The miR-30 family microRNAs confer epithelial phenotype to human pancreatic cells," *Islets*, vol. 1, no. 2, pp. 137–147, 2009.
- [143] O. Bar-Nur, H. A. Russ, S. Efrat, and N. Benvenisty, "Epigenetic memory and preferential lineage-specific differentiation in induced pluripotent stem cells derived from human pancreatic islet beta cells," *Cell Stem Cell*, vol. 9, no. 1, pp. 17–23, 2011.
- [144] M. Brissova, M. J. Fowler, W. E. Nicholson et al., "Assessment of human pancreatic islet architecture and composition by laser scanning confocal microscopy," *Journal of Histochemistry and Cytochemistry*, vol. 53, no. 9, pp. 1087–1097, 2005.
- [145] D. Bosco, M. Armanet, P. Morel et al., "Unique arrangement of α - and β -cells in human islets of Langerhans," *Diabetes*, vol. 59, no. 5, pp. 1202–1210, 2010.
- [146] R. Rodriguez-Diaz, M. H. Abdulreda, A. L. Formoso et al., "Innervation patterns of autonomic axons in the human endocrine pancreas," *Cell Metabolism*, vol. 14, no. 1, pp. 45–54, 2011.
- [147] J. A. Kushner, G. C. Weir, and S. Bonner-Weir, "Ductal origin hypothesis of pancreatic regeneration under attack," *Cell Metabolism*, vol. 11, no. 1, pp. 2–3, 2010.
- [148] L. C. Murtaugh, "Stem cells and β cells: the same, but different?" *Cell Stem Cell*, vol. 8, no. 3, pp. 244–245, 2011.

Review Article

Engraftment of Insulin-Producing Cells from Porcine Islets in Non-Immune-Suppressed Rats or Nonhuman Primates Transplanted Previously with Embryonic Pig Pancreas

Marc R. Hammerman

George M. O'Brien Center for Kidney Disease Research, Departments of Medicine, and Cell Biology and Physiology, The Washington University School of Medicine, St. Louis, MO 63110, USA

Correspondence should be addressed to Marc R. Hammerman, mhammerm@dom.wustl.edu

Received 17 May 2011; Revised 2 July 2011; Accepted 2 July 2011

Academic Editor: Thierry Berney

Copyright © 2011 Marc R. Hammerman. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Transplantation therapy for diabetes is limited by unavailability of donor organs and outcomes complicated by immunosuppressive drug toxicity. Xenotransplantation is a strategy to overcome supply problems. Implantation of tissue obtained early during embryogenesis is a way to reduce transplant immunogenicity. Insulin-producing cells originating from embryonic pig pancreas obtained very early following pancreatic primordium formation (embryonic day 28 (E28)) engraft long-term in non-immune, suppressed diabetic rats or rhesus macaques. Morphologically, similar cells originating from adult porcine islets of Langerhans (islets) engraft in non-immune-suppressed rats or rhesus macaques previously transplanted with E28 pig pancreatic primordia. Our data are consistent with induction of tolerance to an endocrine cell component of porcine islets induced by previous transplantation of embryonic pig pancreas, a novel finding we designate organogenetic tolerance. The potential exists for its use to enable the use of pigs as islet cell donors for humans with no immune suppression requirement.

1. Introduction

We have reviewed previously, for *J. Transplantation*, why transplantation of embryonic pancreatic primordia to replace endocrine pancreas function is advantageous relative to transplantation of either pluripotent embryonic stem (ES) cells, or of terminally differentiated (adult) organs [1]: (1) unlike ES cells, pancreatic primordia differentiate along defined lines without a need to steer differentiation; (2) there is no risk of teratoma formation; (3) the growth potential of cells within embryonic pancreas is enhanced relative to those in the terminally differentiated organ; (4) the cellular immune response to transplanted embryonic pancreas is attenuated relative to that directed against the adult organ; (5) the ability of avascular primordia to attract a host blood supply renders them less susceptible to humoral rejection than is donor-vascularized adult pancreas after transplantation across a discordant xenogeneic barrier; and (6) exocrine pancreatic tissue does not differentiate following

transplantation of embryonic pancreas, obviating inflammatory complications that result from exocrine components.

Transplantation of human embryonic pancreas in human hosts has been contemplated [2]. However, we [3–8] and others [9–12] have focused on the use of embryonic pancreas from the pig, a physiologically suitable donor for humans [13, 14]. Glucose tolerance can be normalized in streptozotocin- (STZ-) diabetic (type 1) LEW [3, 4, 7] rats or ZDF (type 2) diabetic rats [5] within 4 weeks following transplantation in mesentery of pig pancreatic primordia obtained very early during embryogenesis (on embryonic day 28 (E28)—just after the organ differentiates and prior to the time dorsal and ventral anlagen fuse) without host immune suppression. Rats are rendered permanently independent of a requirement for exogenous insulin to maintain normoglycemia. No circulating rat insulin can be detected in STZ-treated rats. Rather, porcine insulin circulates after transplantation of E28 pig pancreatic primordia levels of which increase after a glucose load. Cells with beta

cell morphology expressing insulin and porcine proinsulin mRNA engraft in host mesentery, mesenteric lymph nodes, liver, and pancreas after transplantation.

Cells originating from E28 pig pancreatic primordia transplanted in mesentery engraft similarly in non-immunosuppressed STZ-diabetic rhesus macaques [6, 8]. Glucose tolerance can be nearly normalized in non-immunosuppressed diabetic macaques following transplantation of E28 pig pancreatic primordia [1]. Porcine insulin, but not primate insulin, circulates after transplantation in macaques [6]. Exogenous insulin requirements are reduced in transplanted macaques [6]. Animals have been weaned off insulin for short periods of time, but not permanently [1]. The most likely explanation for the differential success between rats and macaques is that macaques weigh 20 times as much as rats. A STZ-diabetic rat can be rendered normoglycemic lifelong with no exogenous insulin requirement by transplantation of 5–8 pig pancreatic primordia. Extrapolating, it would take 100–200 primordia to render a diabetic macaque independent of exogenous insulin. This would require the sacrifice of about 10–20 pregnant sows and multiple surgeries with the attendant complications [7].

In lieu of increasing the numbers of transplanted primordia or transplant surgeries in diabetic rhesus macaques, we embarked on a series of experiments to determine whether porcine islets, a more easily obtainable and possibly more robust source of insulin-producing cells, could be substituted for animals in which embryonic pig pancreas already had been engrafted. To this end, we implanted adult porcine islets beneath the capsule of one kidney of rats or macaques, that several weeks earlier had been transplanted with E28 pig pancreatic primordia in mesentery. We employed the renal subcapsular site for islet implantation so that we could differentiate engrafted porcine tissue originating from the islets from tissue originating from prior mesenteric E28 pig pancreatic transplants, that never engraft in host kidney [7, 8]. In this setting, the contralateral (nontransplanted) kidney served as a control, as did kidneys from rats or macaques implanted with islets without prior transplantation of E28 pig pancreatic primordia in mesentery [7, 8]. As in experiments demonstrating engraftment of cells originating from E28 pig pancreatic primordia transplanted in mesentery of rats or macaques [3–6], we employed multiple techniques to ascertain whether cells from porcine islets engrafted in kidney: immune histochemistry for insulin; *in situ* hybridization specific for porcine proinsulin mRNA; fluorescent *in situ* hybridization for pig X chromosomes; RT-PCR specific for porcine proinsulin mRNA; measurement of glucose-stimulated insulin release *in vitro* from implanted kidney tissue; electron microscopy [7, 8].

Figures 1(a) and 1(b) show sections from a kidney of a STZ-diabetic rat implanted with porcine islets following transplantation of E28 pig pancreatic primordia in mesentery. Sections are stained using anti-insulin antibodies (Figure 1(a)) or control serum (Figure 1(b)). Cells that stain for insulin (Figure 1(a), arrows), but not with control serum (Figure 1(b)), are present in an expanded renal subcapsular space [7]. Figures 1(c)–1(g) show sections from a kidney of a STZ-diabetic rhesus macaque following transplantation

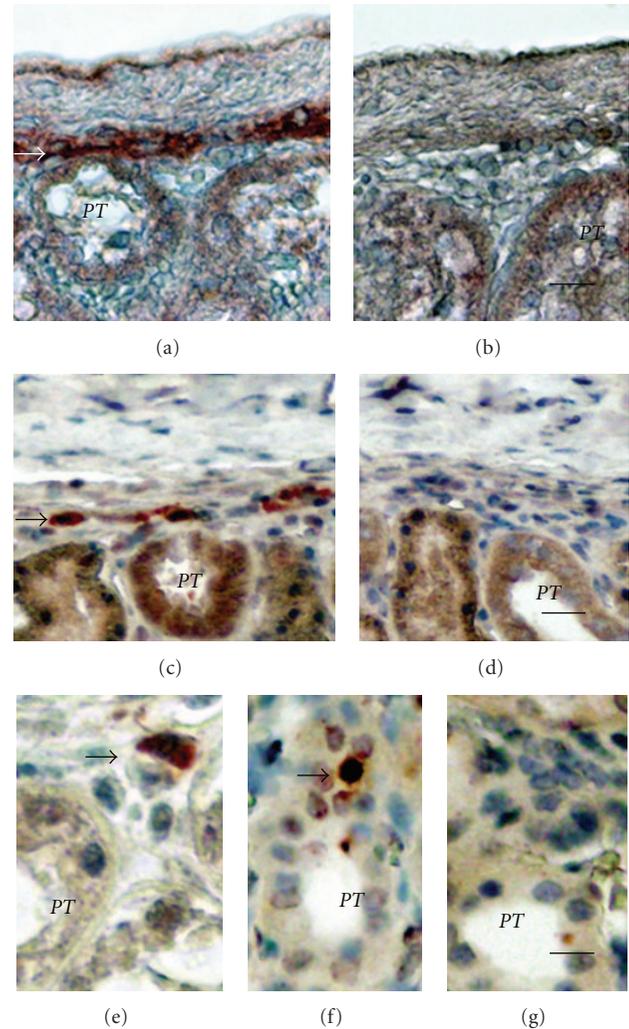


FIGURE 1: Sections of the islet-implanted kidney from a rat (a, b) or rhesus macaque (c–g) transplanted with E28 pig pancreatic primordia in mesentery followed by porcine islets in the renal subcapsular space stained using anti-insulin antibodies (a, c, and e) or control antiserum (b, d); or hybridized to an antisense (f) or sense (g) probe for porcine proinsulin mRNA. PT: proximal tubule. Arrows: positively staining cells (a, c, and e). Scale bars 10 μ m (a, b); 15 μ m (c, d); 7.5 μ m (e–g). Reproduced with permission from the American Society for Investigative Pathology [7] and from Organogenesis [8].

of E28 pig pancreatic primordia in mesentery and subsequent implantation of islets in the kidney. Sections are stained using anti-insulin antibodies (Figures 1(c) and 1(e)) or control serum (Figure 1(d)) or hybridized to an antisense (Figure 1(f)) or sense (Figure 1(g)) probe specific [6] for porcine proinsulin mRNA. As was the case in rats (Figure 1(a)), a row of cells that stain for insulin is present in the subcapsular space (Figure 1(c) arrow). A high-power view of a single insulin-staining cell is shown in Figure 1(e) (arrow). It is polygonal with a round nucleus, a beta cell morphology [8]. No staining for insulin is observed in sections incubated with control antiserum (Figure 1(d)). A

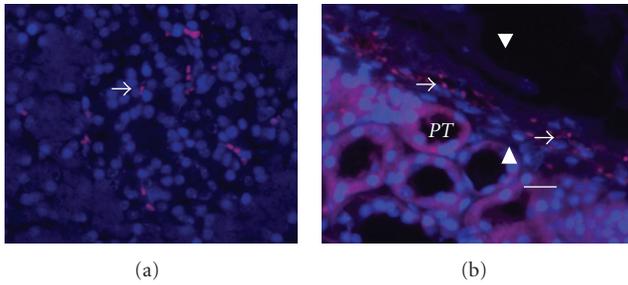


FIGURE 2: Fluorescence microscopy of tissue sections originating from (a) a normal porcine pancreas or (b) a subcapsular section from a kidney of a rhesus macaque transplanted with embryonic pig pancreas in mesentery and subsequently with porcine islets in that kidney. *PT*: proximal tubule, arrows: delineate pig X chromosomes. Arrowheads: renal capsule (b). Scale bar 10 μ m. Reproduced with permission from Organogenesis [8].

cell in the subcapsular space to which the antisense porcine proinsulin mRNA probe binds is shown in Figure 1(f) (arrow). No hybridization is observed if a sense probe is substituted for the antisense probe (Figure 1(g)).

Neither cells that stain for insulin nor cells to which the probe for porcine proinsulin mRNA binds are present in contralateral (nonimplanted) kidneys of STZ diabetic rats [7] or macaques [8] in which E28 pig pancreatic primordia were transplanted previously in mesentery or in kidneys from STZ-diabetic rats [7] or macaques [8] into which porcine islets are implanted without prior transplantation of E28 pig pancreatic primordia in mesentery. Presumably, the implanted tissue is rejected by the host [7, 8].

To provide additional evidence that cells in the kidneys of islet-implanted rats or macaques previously transplanted with E28 pig pancreatic primordia in mesentery are of porcine origin, we demonstrated using fluorescent in situ hybridization, that the cells contain pig X chromosomes [7, 8]. Shown in Figure 2(a) are pig X chromosomes in nuclei of cells from a normal porcine pancreas (positive control). Figure 2(b) shows pig X chromosomes (arrows) in the nuclei of cells in the renal subcapsular space (arrowheads) from a STZ diabetic rhesus macaque transplanted with E28 pig pancreatic primordia in mesentery followed by porcine islets in kidney.

Multiple organs were excised from a STZ-diabetic macaque transplanted with E28 pig pancreatic primordia in mesentery and subsequently with porcine islets in the renal subcapsular space of one kidney. Tissues were homogenized individually and total RNA was purified. RT-PCR was performed using primers specific for pig or monkey proinsulin mRNA. Products were separated by electrophoresis on 3% agarose gels and their identities confirmed by sequencing in the Washington University Core Protein and Nucleic Acid Chemistry Laboratory [8]. Results are shown in Figure 3(a). The pig primers amplify a band of 193 bps in RNA originating from pig pancreas, corresponding to pig proinsulin insulin mRNA. The rhesus macaque (monkey) primers amplify a band of 199 bps corresponding to monkey proinsulin mRNA in monkey pancreas. Pig proinsulin

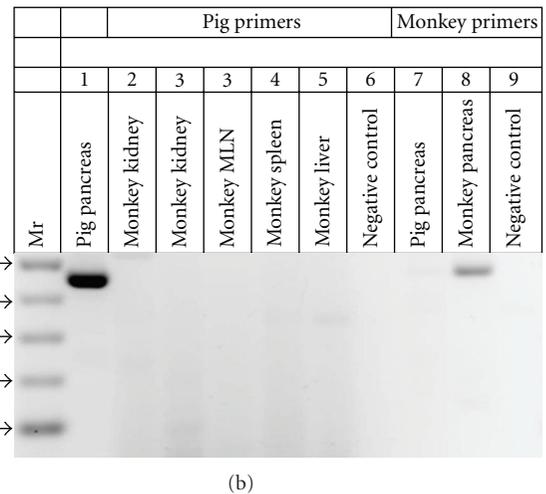
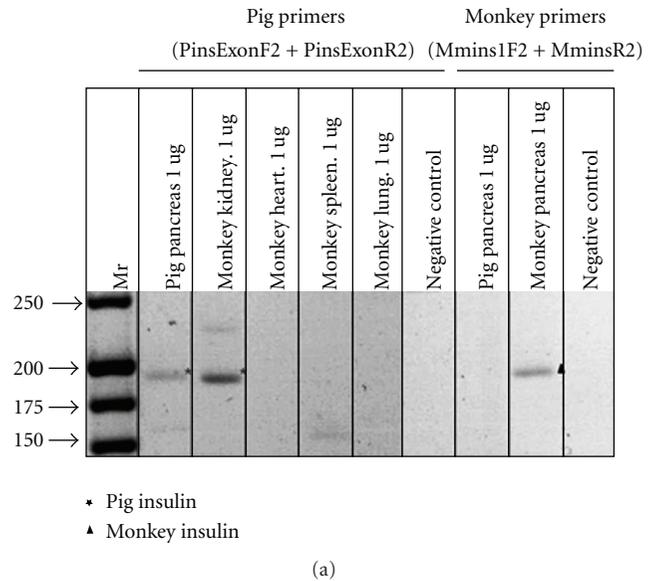


FIGURE 3: RT-PCR: (a) shown left to right are DNA molecular weights (Mr); amplification of bands using primers specific for porcine proinsulin from 1 μ g RNA extracted from pig pancreas or from a rhesus macaque (monkey) transplanted with E28 pig pancreatic primordia in mesentery followed by implantation of porcine islets in the renal subcapsular space: kidney, heart, spleen, lung, a negative control for porcine-specific primers (no RNA); amplification of bands using primers specific for monkey proinsulin from 1 μ g of pig pancreas RNA; monkey pancreas; a second negative control for macaque-specific primers. (b) Shown left to right are DNA molecular weights (Mr); amplification of bands using primers specific for porcine proinsulin from 2 μ g RNA extracted from pig pancreas or from a rhesus macaque (monkey) implanted with porcine islets in the renal subcapsular space with no previous transplantation of E28 pig pancreatic primordia: kidneys, mesenteric lymph node (MLN) spleen, liver, a negative control for porcine-specific primers (no RNA); amplification of bands using primers specific for monkey proinsulin from 2 μ g of pig pancreas RNA; monkey pancreas and a second negative control for macaque-specific primers. Pig primers amplify a 193 bps band. Monkey primers amplify a 199 bps band. Reproduced with permission from Organogenesis [8].

mRNA is also detected in the islet-implanted monkey kidney. Multiple organs were excised from a STZ-diabetic macaque transplanted with porcine islets in the renal subcapsular space of one kidney with no prior transplantation of E28 pig pancreatic primordia in mesentery and RT-PCR performed as above. As shown in Figure 3(b), no pig proinsulin mRNA was detected in any monkey organ including the transplanted kidney.

To ascertain whether cells originating from kidney-implanted porcine islets function in rats or rhesus macaques, we determined whether the glucose tolerance of STZ-diabetic animals normalized partially by prior transplantation of E28 pig pancreatic primordia in mesentery was rendered normal by subsequent islet implantation, and measured glucose-stimulated insulin release from islet-implanted kidneys in vitro. Rats were rendered fully glucose tolerant by subsequent implantation of porcine islets in one kidney [7]. The glucose tolerance of macaques normalized partially by prior transplantation of E28 pig pancreatic primordia in mesentery was not improved by subsequent implantation of islets in kidney [8]. However, a rapid release of insulin by macaque kidney slices was demonstrated in vitro in response to elevation of glucose levels across the threshold for insulin release [8].

As illustrated in a representative of 3 experiments using weight-matched tissue, no insulin could be detected at time 0 in supernatants from the implanted macaque kidney (Figure 4(a)). However, insulin was detectable by 1 min after increasing the glucose level in vitro. No insulin was detected at any time in any supernatants from the nonimplanted kidney (Figure 4(b)), or in supernatants from a kidney of a macaque in which porcine islets were implanted without prior transplantation of E28 pig pancreatic primordia in mesentery [8].

Cells containing endocrine granules in an expanded renal subcapsular space were identified in electron micrographs of kidneys from rats implanted with porcine islets following transplantation of E28 pig pancreatic primordia in mesentery [7]. Figure 5 is an electron micrograph of the subcapsular space from a rhesus macaque kidney into which porcine islets were implanted following transplantation of E28 pig pancreatic primordia in mesentery. Shown is a cell with encapsulated granules (arrows) characteristic of endocrine secretory granules [8].

2. Discussion

The shortage of human pancreas donor organs imposes severe restrictions on the use of allotransplantation to treat diabetes mellitus [15–22]. When performed, whole pancreas transplantation requires use of potent immunosuppressive medications that have significant complications. Newer, more targeted immunosuppressive regimens that do not require steroids or high-dose calcineurin inhibitors make islet transplantation a more attractive option. However, side effects of immune suppression that must be maintained so long as the islet graft functions remain a source of morbidity and even mortality [15]. Thus transplantation therapy for diabetes trades one set of morbidities (associated with

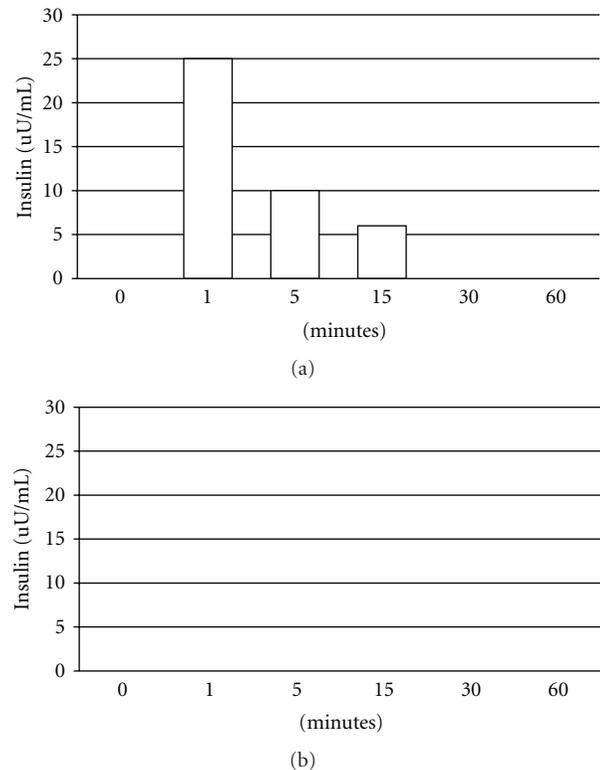


FIGURE 4: Levels of insulin (uU/mL) measured over 60 min in vitro after addition of glucose to tissue following time 0: (a) from a macaque kidney implanted with porcine islets following transplantation of E28 pig pancreatic primordia in mesentery; (b) the contralateral nonimplanted kidney. Reproduced with permission from Organogenesis [8].

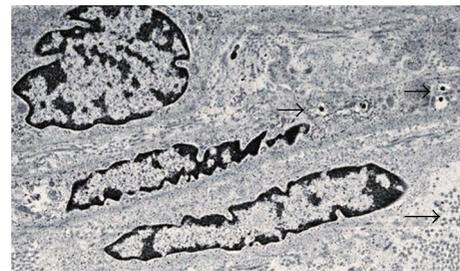


FIGURE 5: Electron micrograph of rhesus macaque kidney following sequential transplantation of E28 pig pancreatic primordia in mesentery and implantation of porcine islets in the kidney. Arrows: endocrine granules. Scale bar a 2 μ m. Reproduced with permission from Organogenesis [8].

diabetes and its medical treatment) for another (associated with immune suppression).

The severity of humoral rejection effectively precludes the use of pigs as whole pancreas organ donors for humans. However, because they are vascularized by the host posttransplantation, islets like other cell transplants are not subject to humoral rejection. Porcine islets are rejected within two weeks of transplantation in non-immune suppressed non-human primates [8, 16–18]. Experience

with pig to primate islet or neonatal islet transplantation in immune suppressed non-human primates shows that sustained insulin independence can be achieved, but only through the use of agents that are not approved for human use or that result in a high level of morbidity and mortality [19–21]. Thus, the need for host immune suppression is a barrier for pig-to-human islet xenotransplantation.

Xenotransplantation of embryonic pig pancreatic primordia in lieu of mature pig organs or porcine islets couples the wide availability of pig organs with the immunological advantages inherent in transplanting cellular embryonic tissue, circumventing humoral rejection and obviating the need for host immune suppression [1]. However, obtaining embryonic pig pancreata is technically challenging because surgery must be performed on multiple pregnant sows and isolation carried out from scores of embryos to obtain sufficient numbers of primordia. Furthermore, transplanting pancreatic primordia in mesentery of primates is invasive, requiring that a host laparotomy be performed on one or more occasions. In contrast, porcine islets can be isolated in large quantities from a single pig pancreas, and infusion of porcine islets can be carried out via the portal vein infusion without a laparotomy [22].

Cells from porcine islets do not survive after implantation in rat or macaque kidneys without prior transplantation of E28 pig pancreatic primordia transplantation in mesentery [7, 8]. Whole porcine islets do not engraft in kidneys. Rather, an endocrine (beta cell) component originating from porcine islets does so [7, 8]. Ours is the first report describing sustained survival of such cells following transplantation of porcine islets in non-immune-suppressed primates. Glucose tolerance in diabetic rats not fully normalized by prior transplantation of E28 pig pancreatic primordia in mesentery is corrected following subsequent implantation of porcine islets in kidney [7]. In contrast, correction is not observed following E28 pig pancreatic primordia transplantation and porcine islets implantation in macaques [8]; that is, consistent with previous observations that glucose tolerance is more difficult to correct in macaques than in rats [6]. It is possible that the cell component, although of sufficient mass to normalize glucose tolerance in diabetic rats following implantation of islets [7], is insufficient following implantation in rhesus macaques [8]. Implantation of more islets (isolated from more than one adult pig pancreas) in kidney or infusion of porcine islets into a site from which insulin can act more directly on liver (the portal vein) [22] following transplantation of E28 pig pancreatic primordia in mesentery may be a better way to normalize glucose tolerance. Alternatively, it may be that the mass of engrafted cells originating from porcine islets implanted in kidney (perhaps derived from a numerically small stem cell component within the islets) [7, 8] is insufficient to impact on control of circulating glucose in an animal as large as a macaque.

Schroeder et al. [23] define transplantation tolerance as immune unresponsiveness to the transplanted organ, but not to other antigens in the absence of ongoing immunosuppression. Lewis rats transplanted with E28 pig pancreatic primordia retain reactivity to other porcine xenoantigens

(E28 pig renal primordia are rejected [4]). Thus, our findings are consistent with induction of specific tolerance [23] to a cell component (either beta cells or a stem cell component that differentiates into insulin-producing cells) of adult porcine islets implanted in Lewis rats by previous transplantation of E28 pig pancreatic primordia.

Engraftment of pancreatic progenitors transplanted across a xenogeneic barrier to non-immune-suppressed immune-sufficient hosts has been reported twice previously. Eloy et al. described normalization of glucose posttransplantation of E15, but not E18 embryonic chick pancreas into liver of non-immune-suppressed STZ-diabetic rats [24]. Abraham et al. [25] described successful xenoengraftment in multiple organs of human pancreatic islet-derived progenitor cells infused in nonimmunosuppressed mice. Neither Eloy et al. [24] and Abraham et al. [25] nor we [3–8] define an immunological mechanism for the finding. Although the antigenicity of fetal tissues may be less than that of corresponding adult tissues, animal data suggest the reduction is not enough by itself to ensure permanent graft survival [26]. Thus, the use of embryonic tissue (pancreas) per se cannot explain the results.

Host immune suppression is required for successful engraftment of embryonic pig pancreas in rodents [11] or non-human primates [12] carried out using methodology alternative to ours. Therefore, it is likely that one or more factors in the methodology we employ [3–5, 7], different from that used by others (the protocol of Tchorsh-Yutsis et al.) [11], are critical for engraftment without an immune suppression requirement. Such factors include those listed in Table 1 for studies that employ rats as hosts for pancreatic primordia. First, the developmental stage of donor pig embryos from which primordia are obtained impacts on the host immune response. We have shown that E35 pig pancreatic primordia are rejected in Lewis rats following transplantation, employing conditions under which E28 or E29 pig pancreatic primordia are engrafted [4]. While we have no experience transplanting E42 pig pancreatic primordia in rats, the preferred stage for studies described by Tchorsh-Yutsis et al. [11], we would expect them to reject based on our experience with E35 pancreatic primordia [4]. Second, it is likely that that incubation of embryonic pancreas prior to transplantation with one or more growth factors and cytokines (iron-saturated transferrin; prostaglandin E1 and vascular endothelial growth factor; hepatocyte growth factor) [3–5, 7] alters the host immune response. Tchorsh-Yutsis et al. do not employ such agents [11]. Third, it is possible that hyperglycemia in diabetic hosts [3–5, 7] impairs the immune response to embryonic pancreas. Tchorsh-Yutsis et al. transplant pancreatic primordia into nondiabetic rats [11]. Fourth, the transplantation site and technique probably impacts on the host immune response. We interpose pancreatic primordia between sheets of mesentery [3–5, 7]. Tchorsh-Yutsis et al. transplant into pockets of omentum and secure using suture [11], the latter in itself likely to trigger inflammation. We have proposed [27] that transplantation of E28 pig pancreatic primordia in the mesentery and migration of cells to mesenteric lymph nodes and liver recapitulates events

TABLE 1: Methodology employed for transplantation of embryonic pig pancreas in immune sufficient non-immune-suppressed rats.

	References [3–5, 7]	Reference [11]
Developmental stage of donors	E28 preferred, E29	E28, E42 preferred
Incubation prior to transplantation	Growth factors & cytokines	None
Diabetic status of the host	Diabetic	Nondiabetic
Transplantation site	Interposed within mesentery	Sutured in omentum

that occur during induction of oral tolerance [28–30], which is dependent on antigen transport via afferent lymphatics into the draining mesenteric lymph nodes [30]. In effect, we suggest that heterotopic introduction of embryonic pig pancreas in rat or primate mesentery coopts the function of the gut-associated lymphoid tissues (GALT), a complex, redundant [28–30], and phylogenetically ancient system [31, 32] of which embryonic pancreas is a part [33], that, under normal conditions, induces peripheral tolerance to ingested antigens in jawed vertebrates and their descendants.

Interestingly, GALT may have served similarly to prevent an immune response to insulin-producing cells scattered originally in the gut epithelium of primitive vertebrates [31, 32] and has been proposed to induce tolerance or immune suppression towards islet cell antigens during normal embryonic development [33]. Developmentally controlled lymphogenesis establishes a preferential trafficking route from the gut to pancreatic lymph nodes, a GALT component, in which T cells can be activated by antigens drained from the peritoneum and the gastrointestinal tract. Intestinal stress modifies the presentation of pancreatic self-antigens in pancreatic lymph nodes. The convergence of endocrine and intestinal contents at this site may explain the link between an autoimmune pathogenesis for type 1 diabetes and environmental provocation [33, 34]. Low doses of orally administered antigen induce antigen-specific peripheral tolerance through active suppression of T cells and induction of clonal anergy. High doses induce tolerance by extrathymic deletion of antigen-reactive T cells [35]. It was proposed originally that oral tolerance depends exclusively on antigen uptake by cells within intestinal Peyer’s Patches [30]. However, recently it has been shown that high-dose oral tolerance can be induced in the absence of Peyer’s Patches so long as mesenteric lymph nodes are present [30, 36].

Harada et al. have proposed a similar coopting of oral tolerance to explain the muted immune response in vivo and by cells from mesenteric lymph nodes in vitro to a colon carcinoma of BALB/c origin or a human CD80-transfected DBA/2 mastocytoma injected into the subserosal space of cecum in BALB/c mice relative to tumors injected subcutaneously [37].

One way to confirm a causative link between gut immunity and our ability to transplant E28 pig pancreatic primordia and porcine islets in non-immune-suppressed hosts would be to “break” the established oral tolerance [38], using glucose control as a readout in the rat model, theoretically possible so long as extrathymic deletion of antigen-reactive T cells [35] has not occurred.

In any case, we have demonstrated in two species [7, 8], a novel finding that prior transplantation of embryonic tissue (pancreas) enables engraftment of a cell component from differentiated adult tissue from the same organ (islets) transplanted subsequently, without the need for host immune suppression, a phenomenon, the immunologic mechanism for which remains undefined, that we term organogenetic tolerance [27]. Applicability of the finding to organ replacement therapy in humans awaits definition. The potential exists for its use to enable the use of pigs as islet cell donors for humans with a need for no immune suppression.

Acknowledgment

M. R. Hammerman is supported by P30 DK079333 from NIDDK.

References

- [1] M. R. Hammerman, “Xenotransplantation of embryonic pig kidney or pancreas to replace the function of mature organs,” *Journal of Transplantation*, Article ID 501749, 2011.
- [2] K. Brands, E. Colvin, L. J. Williams, R. Wang, R. B. Lock, and B. E. Tuch, “Reduced immunogenicity of first-trimester human fetal pancreas,” *Diabetes*, vol. 57, no. 3, pp. 627–634, 2008.
- [3] S. A. Rogers, F. Chen, M. Talcott, and M. R. Hammerman, “Islet cell engraftment and control of diabetes in rats after transplantation of pig pancreatic anlagen,” *American Journal of Physiology*, vol. 286, no. 4, pp. E502–E509, 2004.
- [4] S. A. Rogers, H. Liapis, and M. R. Hammerman, “Normalization of glucose post-transplantation of pig pancreatic anlagen into non-immunosuppressed diabetic rats depends on obtaining anlagen prior to embryonic day 35,” *Transplant Immunology*, vol. 14, no. 2, pp. 67–75, 2005.
- [5] S. A. Rogers, F. Chen, M. Talcott, H. Liapis, and M. R. Hammerman, “Glucose tolerance normalization following transplantation of pig pancreatic primordia into non-immunosuppressed diabetic ZDF rats,” *Transplant Immunology*, vol. 16, no. 3–4, pp. 176–184, 2006.
- [6] S. A. Rogers, F. Chen, M. R. Talcott et al., “Long-term engraftment following transplantation of pig pancreatic primordia into non-immunosuppressed diabetic rhesus macaques,” *Xenotransplantation*, vol. 14, no. 6, pp. 591–602, 2007.
- [7] S. A. Rogers, T. Mohanakumar, H. Liapis, and M. R. Hammerman, “Engraftment of cells from porcine islets of Langerhans and normalization of glucose tolerance following transplantation of pig pancreatic primordia in nonimmunosuppressed diabetic rats,” *American Journal of Pathology*, vol. 177, no. 2, pp. 854–864, 2010.

- [8] S. A. Rogers, R. Tripathi, T. Mohanakumar et al., "Engraftment of cells from porcine islets of Langerhans following transplantation of pig pancreatic primordia in non-immune suppressed diabetic rhesus macaques," *Organogenesis*, vol. 7, no. 3, 2011.
- [9] S. Eventov-Friedman, H. Katchman, E. Shezen et al., "Embryonic pig liver, pancreas, and lung as a source for transplantation: optimal organogenesis without teratoma depends on distinct time windows," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 8, pp. 2928–2933, 2005.
- [10] S. Eventov-Friedman, D. Tchorsh, H. Katchman et al., "Embryonic pig pancreatic tissue transplantation for the treatment of diabetes," *PLoS Medicine*, vol. 3, no. 7, pp. 1165–1177, 2006.
- [11] D. Tchorsh-Yutsis, G. Hecht, A. Aronovich et al., "Pig embryonic pancreatic tissue as a source for transplantation in diabetes: transient treatment with anti-LFA1, anti-CD48, and FTY720 enables long-term graft maintenance in mice with only mild ongoing immunosuppression," *Diabetes*, vol. 58, no. 7, pp. 1585–1594, 2009.
- [12] G. Hecht, S. Eventov-Friedman, C. Rosen et al., "Embryonic pig pancreatic tissue for the treatment of diabetes in a nonhuman primate model," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 21, pp. 8659–8664, 2009.
- [13] D. H. Sachs, "The pig as a potential xenograft donor," *Veterinary Immunology and Immunopathology*, vol. 43, no. 1–3, pp. 185–191, 1994.
- [14] Z. Ibrahim, J. Busch, M. Awwad, R. Wagner, K. Wells, and D. K. C. Cooper, "Selected physiologic compatibilities and incompatibilities between human and porcine organ systems," *Xenotransplantation*, vol. 13, no. 6, pp. 488–499, 2006.
- [15] E. Hathout, J. Lakey, and J. Shapiro, "Islet transplant: an option for childhood diabetes?" *Archives of Disease in Childhood*, vol. 88, no. 7, pp. 591–595, 2003.
- [16] D. Dufrane, R. M. Goebels, A. Saliez, Y. Guiot, and P. Gianello, "Six-month survival of microencapsulated pig islets and alginate biocompatibility in primates: proof of concept," *Transplantation*, vol. 81, no. 9, pp. 1345–1353, 2006.
- [17] N. Kirchof, S. Shibata, M. Wijkstrom et al., "Reversal of diabetes in non-immunosuppressed rhesus macaques by intraportal porcine islet xenografts precedes acute cellular rejection," *Xenotransplantation*, vol. 11, no. 5, pp. 396–407, 2004.
- [18] D. Cantarovich, G. Blanche, N. Potiron et al., "Rapid failure of pig islet transplantation in non human primates," *Xenotransplantation*, vol. 9, no. 1, pp. 25–35, 2002.
- [19] B. J. Hering, M. Wijkstrom, M. L. Graham et al., "Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates," *Nature Medicine*, vol. 12, no. 3, pp. 301–303, 2006.
- [20] K. Cardona, G. S. Korbitt, Z. Milas et al., "Long-term survival of neonatal porcine islets in nonhuman primates by targeting costimulation pathways," *Nature Medicine*, vol. 12, no. 3, pp. 304–306, 2006.
- [21] D. J. van der Windt, R. Bottino, A. Casu et al., "Long-term controlled normoglycemia in diabetic non-human primates after transplantation with hCD46 transgenic porcine islets," *American Journal of Transplantation*, vol. 9, no. 12, pp. 2716–2726, 2009.
- [22] D. J. van der Windt, G. J. Echeverri, J. N. M. Ijzermans, and D. K. C. Cooper, "The choice of anatomical site for islet transplantation," *Cell Transplantation*, vol. 17, no. 9, pp. 1005–1014, 2008.
- [23] R. A. Schroeder, C. E. Marroquin, and P. C. Kuo, "Tolerance and the "Holy Grail" of transplantation," *Journal of Surgical Research*, vol. 111, no. 1, pp. 109–119, 2003.
- [24] R. Eloy, K. Haffen, M. Kedinger, and J. F. Grenier, "Chick embryo pancreatic transplants reverse experimental diabetes of rats," *Journal of Clinical Investigation*, vol. 64, no. 2, pp. 361–373, 1979.
- [25] E. J. Abraham, S. Kodama, J. C. Lin, M. Ubeda, D. L. Faustman, and J. F. Habener, "Human pancreatic islet-derived progenitor cell engraftment in immunocompetent mice," *American Journal of Pathology*, vol. 164, no. 3, pp. 817–830, 2004.
- [26] A. A. Rossini, D. L. Greiner, and J. P. Mordes, "Induction of immunologic tolerance for transplantation," *Physiological Reviews*, vol. 79, no. 1, pp. 99–141, 1999.
- [27] M. R. Hammerman, "Organogenetic tolerance," *Organogenesis*, vol. 6, no. 4, pp. 270–275, 2010.
- [28] A. J. Macpherson and K. Smith, "Mesenteric lymph nodes at the center of immune anatomy," *Journal of Experimental Medicine*, vol. 203, no. 3, pp. 497–500, 2006.
- [29] I. N. Crispe, "Hepatic T cells and liver tolerance," *Nature Reviews Immunology*, vol. 3, no. 1, pp. 51–62, 2003.
- [30] T. Worbs, U. Bode, S. Yan et al., "Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells," *Journal of Experimental Medicine*, vol. 203, no. 3, pp. 519–527, 2006.
- [31] T. Matsunaga and A. Rahman, "In search of the origin of the thymus: the thymus and GALT may be evolutionarily related," *Scandinavian Journal of Immunology*, vol. 53, no. 1, pp. 1–6, 2001.
- [32] J. H. Youson and A. A. Al-Mahrouki, "Ontogenetic and phylogenetic development of the endocrine pancreas (islet organ) in fishes," *General and Comparative Endocrinology*, vol. 116, no. 3, pp. 303–335, 1999.
- [33] A. Jansen, H. A. M. Voorbij, P. H. M. Jeucken, G. J. Bruining, H. Hooijkaas, and H. A. Drexhage, "An immunohistochemical study on organized lymphoid cell infiltrates in fetal and neonatal pancreases. A comparison with similar infiltrates found in the pancreas of a diabetic infant," *Autoimmunity*, vol. 15, no. 1, pp. 31–38, 1993.
- [34] S. J. Turley, J. W. Lee, N. Dutton-Swain, D. Mathis, and C. Benoist, "Endocrine self and gut non-self intersect in the pancreatic lymph nodes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 49, pp. 17729–17733, 2005.
- [35] Y. Chen, J. I. Inobe, R. Marks, P. Gonnella, V. K. Kuchroo, and H. L. Weiner, "Peripheral deletion of antigen-reactive T cells in oral tolerance," *Nature*, vol. 376, no. 6536, pp. 177–180, 1995.
- [36] T. W. Spahn, H. L. Weiner, P. D. Rennert et al., "Mesenteric lymph nodes are critical for the induction of high-dose oral tolerance in the absence of Peyer's patches," *European Journal of Immunology*, vol. 32, no. 4, pp. 1109–1113, 2002.
- [37] M. Harada, K. Matsunaga, Y. Oguchi et al., "The involvement of transforming growth factor β in the impaired antitumor T-cell response at the gut-associated lymphoid tissue (GALT)," *Cancer Research*, vol. 55, no. 24, pp. 6146–6151, 1995.
- [38] M. Flanagan, S. Jain, and J. G. Michael, "Breaking of oral tolerance by an encapsulated antigen," *Annals of the New York Academy of Sciences*, vol. 778, pp. 373–375, 1996.

Review Article

Pancreas Procurement and Preservation for Islet Transplantation: Personal Considerations

Hirofumi Noguchi

Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700-8558, Japan

Correspondence should be addressed to Hirofumi Noguchi, noguch-h@cc.okayama-u.ac.jp

Received 18 May 2011; Revised 29 June 2011; Accepted 13 July 2011

Academic Editor: Antonello Pileggi

Copyright © 2011 Hirofumi Noguchi. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Pancreatic islet transplantation is a promising option for the treatment of type 1 diabetic patients. After the successful demonstration of the Edmonton protocol, islet transplantation has advanced significantly on several fronts, including improved pancreas procurement and preservation systems. Since we frequently use pancreata from donors after cardiac death in Japan, we have applied the *in situ* regional organ cooling system for pancreas procurement to reduce the warm ischemic time. To reduce the apoptosis of pancreatic tissue during cold preservation, we have applied the ductal injection of preservation solution. For pancreas preservation, we use modified Kyoto solution, which is advantageous at trypsin inhibition and less collagenase inhibition. In this paper, we show pancreas procurement and preservation in our group for islet transplantation.

1. Introduction

Diabetes mellitus is a devastating disease, and over 200 million people are affected worldwide, thus representing about 6% of the world population. Type 1 diabetes results from the autoimmune-mediated destruction of insulin-secreting β cells in the islets of Langerhans of the pancreas. Pancreatic islet transplantation represents a viable option for the treatment of patients with unstable type 1 diabetes mellitus with frequent severe hypoglycemia and hypoglycemia unawareness [1, 2]. Recent advances in islet transplantation, including the utilization of donors after cardiac death (DCD) [3–6], single-donor islet transplantation [7–10], and living-donor islet transplantation [11], were based on advanced pancreas transport systems [9, 12, 13], improved islet isolation methods [14–17], enhanced islet engraftment [18–21], and revised immunosuppressant protocols [6, 14, 22]. One of the most important issues affecting islet transplantation is concerned with donor quality [23]. Several critical donor factors have been identified, including donor age, body mass index (BMI), cause of death, usage of vasopressor, hypotensive episode, length of hospitalization, blood glucose levels, transaminases level, creatinine levels, cold preservation time, and procurement team [23–26]. Therefore, effective pancreas procurement and preservation are important for successful

islet isolation and transplantation. In this paper, the current advances in pancreas procurement and preservation for islet transplantation in our group are described.

2. Pancreas Procurement

Pancreata from donors with brain death (DBD) are procured using a standardized technique to minimize warm ischemia. A preservation solution, such as the University of Wisconsin (UW) solution, is used for *in situ* perfusion of the donor. The human pancreas is excised immediately after the liver and before the kidneys. The first and fourth portions of the duodenum are first divided with a 55 mm linear cutter. The attachment between the retroperitoneal portion and the body of the pancreas is then dissected toward the spleen. The superior surface of the pancreas is divided toward the spleen, and the short gastric arteries and vein are dissected until the stomach is separated from the spleen. The pancreas is then rapidly excised *en bloc* together with the spleen. The spleen and duodenum are subsequently removed on a back table, and a cannula is inserted into the main pancreatic duct.

Currently, only a few clinical studies have reported that islet transplantation from DCD is possible to treat type 1 diabetes [27, 28]. Vasopressors are used for most

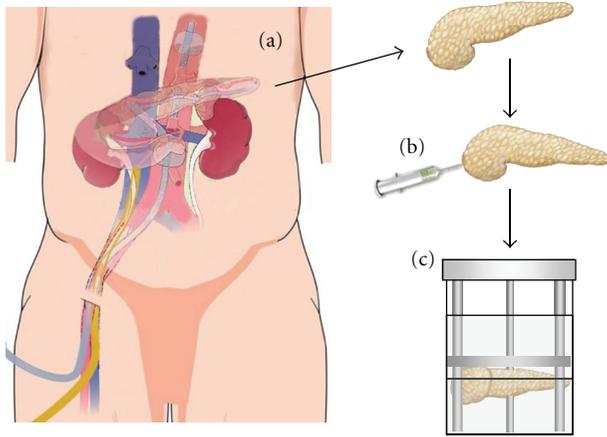


FIGURE 1: Pancreas procurement and preservation for islet transplantation. (a) *In situ* regional cooling system to DCD pancreata. (b) Ductal injection of preservation solution. (c) Pancreas preservation by MK solution/PFC two-layer method.

DCDs, and they tend to have hypotensive episodes, long term hospitalization, and high levels of blood glucose, transaminases, and creatinine which have been identified as critical factors that affect the quality of the pancreas [23–26]. Islet transplantation from DCD is particularly important for countries such as Japan, where the isolation of islets from pancreata of donors who are classified as brain-dead but whose hearts are beating is prohibited by law. We developed the novel procurement technique in collaboration with the kidney procurement team [29]. After confirmation of brain death, a double-balloon catheter is inserted to prevent ischemic damage to the human pancreas by using an *in situ* regional organ cooling (ISRC) system that was originally developed for procurement of the kidney [30]. Before cardiac arrest, a tip of the double-balloon catheter is placed above the celiac axis in the aorta via the femoral artery and only a few centimeters above the location used for an ordinary nephrectomy for procurement from a DCD. A venous catheter is also placed in the inferior vena cava via the femoral vein for drainage of the perfusate and blood. ISRC for the pancreas and kidney (ISRC-PK) is achieved by pump or drip infusion (drip speed 20 mL/min) of a hypothermic lactated ringer solution beginning immediately after cardiac arrest and then continuing until the end of the nephrectomy and pancreatectomy. After laparotomy, the lesser sac is opened by dividing the gastrocolic and gastrohepatic ligaments to determine whether the pancreas has uniform perfusion efficacy by means of ISRC-PK. Perfusion of the pancreas is evaluated by the uniform color change of the pancreas and the coldness of the pancreas surface after laparotomy. After a visual check of the pancreas, 500 mL of sterile crushed ice is placed on it to avoid warm ischemic injury and then the pancreas is harvested (Figure 1(a)) [29].

The ISRC system was originally developed for the procurement of the kidney [30], and the only modification we made is the position of the double-balloon catheter to ensure both pancreas and kidney protection. This ISRC

system reduced the warm ischemic time to only 3 minutes on average [29]. We have used lactate ringer solution instead of UW solution for perfusion. Lactate ringer solution has a low potassium concentration and low viscosity in comparison to UW solution. A low potassium concentration could prevent potassium-induced vasospasms while a low viscosity helps to induce rapid perfusion. Therefore, using lactate ringer for perfusion might be important in ISRC.

3. Ductal Injection of Preservation Solution

We previously developed a new method for large-scale porcine islet isolation from market-weight pigs [31], based on a report by O’Neil et al. [32]. Although some steps of the new method seemed technically inferior to the standard automated method using a Ricordi chamber, islet yield per gram for our new method was relatively higher (but not significantly so) than that for the Ricordi method [33]. It is possible that the advantage of the new method was the injection of 1.0–1.5 mL/g pancreas of UW-D (UW solution with high Na^+ /low K^+) solution. It was also shown that islet yields from pancreata with intraductal flush, along with collagenase prior to preservation, were superior to vascular flush [34]. We speculate that the ductal injection of a large volume of preservation solution (1 mL/g pancreas) may improve the islet yield. We investigated whether ductal injection (UW and modified Kyoto (MK) solution) before pancreas storage improves the islet yields in islet isolation using porcine pancreata. After obtaining the pancreas, we immediately inserted a cannula into the main pancreatic duct, infused a large amount of preservation solution (1 mL/g pancreas) for ductal protection, and placed the pancreas into a preservation container (Figure 1(b)). The islet yield both before and after purification was significantly higher in the ductal injection group than in the control group. The TUNEL-positive cells in the ductal injection group significantly decreased in comparison to the control group. The ductal injection of preservation solution increased the ATP level in the pancreas tissue and reduced trypsin activity during the digestion step. In a transplant model, ductal injection improved the islet graft function. These findings suggest that the ductal injection of preservation solution leads to improved outcomes for pancreatic islet transplantation [35]. Another group also showed the ductal injection of a small volume (0.05–0.1 mL/g) of UW solution at the time of pancreas procurement to improve the islet yield and function in a rodent model [36]. Based on these data, we now use this technique for clinical islet transplantation. With this technique, we rarely see clumping or DNA release, even using DCD, and we never use DNase.

4. Preservation Solution

UW solution has been recognized as the gold standard solution for organ preservation. UW solution is used extensively as a cold storage solution during procurement and transport of the pancreas prior to islet isolation. However,

UW solution has several disadvantages: it must be stored in the cold until use, and its short shelf life makes it expensive. It is also highly viscous, which may complicate the initial organ flush [37]. For islet isolation, it has been observed that UW inhibits the collagenase digestion phase of islet isolation, thus resulting in poor islet yields and islets of poor viability [38, 39]. It has been reported that the components in UW solution found to be most inhibitory were magnesium, low Na^+ /high K^+ , hydroxyethyl starch (HES), and adenosine. Furthermore, previous reports also indicated that allopurinol in combination with either lactobionate or glutathione markedly inhibited collagenase and that the most inhibitory solution tested was a combination of three components, raffinose, glutathione, and lactobionate [39]. We evaluated the effect of MK solution for islet isolation [12]. Kyoto University developed the ET-Kyoto solution, and its effectiveness in cold lung storage has been demonstrated in clinical lung transplantation [40, 41]. It also is effective for skin flap storage, and its clinical application is beginning in this field [42]. MK solution is a modified ET-Kyoto solution, in which ulinastatin is added. MK solution contains trehalose, gluconate, and ulinastatin as distinct components. Trehalose has a cytoprotective effect against stress, and gluconate acts as an extracellular oncotic agent, which prevents cells from swelling [44]. Ulinastatin is a trypsin inhibitor and eliminates trypsin activity during pancreas preservation [12]. Due to the chemical stability of the effective components and other ingredients, MK solution, but not UW solution, can be stored at room temperature for a long period. MK solution has high Na^+ /low K^+ , and it includes only HES at a lower concentration than UW solution, thus suggesting a lower collagenase inhibition than UW solution. It has also been shown that the Na^+ / K^+ ratio, adenosine, allopurinol, and glutathione are not essential for the cold storage of pancreatic digest prior to islet purification [45]. Moreover, trehalose and ulinastatin inhibit collagenase digestion less than UW solution [12]. The high potassium concentration in UW solution causes vasospasms and insulin release from pancreatic β cells [46], and the high viscosity of UW solution may thus prevent sufficient flushing and ductal injection. In both porcine and human islet isolation, the islet yield was significantly higher in the MK group compared with the UW group [12, 47]. These findings show that MK solution is a more effective cold-storage solution in pancreas preservation for islet isolation than UW solution.

We next compared histidine-tryptophan-ketoglutarate (HTK) solution and MK solution for islet isolation. HTK solution was originally developed for cardioplegia and is being used with increasing frequency in cardiac, renal, and hepatic transplantation [48, 49]. The protective effect of HTK solution is based on the strong buffering capacity of histidine. This solution has a low viscosity, easy handling properties, and a relatively low cost. Some studies have demonstrated similar results between UW and HTK solutions for pancreas preservation, in not only experimental animal models [43, 50, 51] but also clinical pancreas transplantation [52–54]. We used HTK solution with ulinastatin (modified HTK solution (M-HTK)) in this study because

MK solution includes ulinastatin. In porcine islet isolation, the islet yield after purification was significantly greater in the MK group than in the M-HTK group. The MK group had a significantly higher ATP level in the islets than in the M-HTK group. These data suggest that MK solution is better for pancreas preservation before islet isolation than M-HTK solution [55]. The M-HTK solution includes magnesium but does not include HES, adenosine, allopurinol, lactobionate, glutathione, or raffinose. There are no significant differences between the MK and M-HTK solutions regarding collagenase activity. Therefore, the different islet yields after purification are not due to differences in collagenase inhibition between these two solutions. Since a significantly higher ATP level in islets was observed in the MK group compared to the M-HTK group, the cytoprotective effect such as HES and/or trehalose might be a factor in the islet yield differences observed between the two solutions. Another group recently reported that, compared with UW solution, HTK solution has similar efficiency for preserving human pancreata for subsequent islet isolation during <10 h cold ischemia time, but prolonged cold storage resulted in a reduced islet yield [56].

Recently, Celsior solution has been used as an alternative solution for organ preservation. Celsior is an extracellular solution deprived of colloid and was initially developed for heart preservation [57]. Preliminary clinical studies showed no differences between UW and Celsior for lung [58], liver [59], and kidney [60] preservation. Hubert et al. recently reported on the application of Celsior solution for *in situ* perfusion of the donor before human and pig islet isolation [61]. Their data showed the *in situ* perfusion of UW solution to be superior to Celsior solution. In contrast to UW, Celsior induced cell swelling and pancreas edema after only four hours of cold storage. The components of Celsior solution are in part similar to MK solution (a high-sodium/low-potassium composition with comparatively low viscosity) and in part similar to UW solution (including lactobionate acid and glutathione). We next compared modified Celsior solution (Celsior solution with HES and nafamostat mesilate, HNC) and MK solution [47]. Since Celsior solution lacks HES, which is an oncotic agent and protects cell swelling, we added HES to Celsior solution in this study. We also added nafamostat mesilate, one of the trypsin inhibitors, to Celsior because one of the advantages of MK solution is trypsin inhibition by ulinastatin. Nafamostat mesilate has a higher level of trypsin inhibition than ulinastatin [62, 63]. In human islet isolation, the islet yield after purification was significantly higher in the MK group than in the HNC group. The HNC group had a longer phase I period (digestion time), a higher volume of undigested tissue, and a higher percentage of embedded islets, thus suggesting that the solution may inhibit collagenase. However, there was no significant difference in the ATP content in the pancreata or in the attainability of posttransplant normoglycemia in diabetic nude mice between the two groups, thus suggesting that the quality of islets was similar between the two groups. These data suggest that MK solution is better for pancreas preservation before islet isolation than HNC solution (Tables 1 and 2).

TABLE 1: Composition and other characteristics of each preserving solution.

	ET-K	MK	UW	HNC	M-HTK
Na (mmol/L)	100	100	29	100	15
K (mmol/L)	43.5	43.5	125	15	10
Mg (mmol/L)	—	—	5	13	4
Ca (mmol/L)	—	—	—	0.25	0.015
Cl (mmol/L)	—	—	—	41	50
Gluconate (mmol/L)	100	100	—	—	—
Phosphate (mmol/L)	25	25	25	—	—
Sulfate (mmol/L)	—	—	5	—	—
Lactobionate (mmol/L)	—	—	100	80	—
Raffinose (mmol/L)	—	—	30	—	—
Trehalose (mmol/L)	120	120	—	—	—
Adenosine (mmol/L)	—	—	5	—	—
Alloprinol (mmol/L)	—	—	1	—	—
Glutathione (mmol/L)	—	—	3	3	—
HES (g/L)	30	30	50	30	—
Ulinastatin ($\times 10^3$ U/L)	—	100	—	—	100
Nafamostat mesilate (mg/L)	—	—	—	20	—
Histidine (mmol/L)	—	—	—	30	198
Mannitol (mmol/L)	—	—	—	60	30
α -ketoglutarate (mmol/L)	—	—	—	—	1
Tryptophan (mmol/L)	—	—	—	—	2
Glutamic acid (mmol/L)	—	—	—	20	—
Osmolality (mOsm)	366	366	320	355	310

HES: hydroxyethyl starch; ROS: reactive oxygen species; ET-K: ET-Kyoto solution; MK: modified ET-Kyoto solution; UW: University of Wisconsin solution; HNC: Celsior solution with HES and nafamostat mesilate; M-HTK: modified histidine-tryptophan-ketoglutarate solution.

TABLE 2: Comparison between the different preservation solutions.

Comparison	Superior	Human/animal study	Reference
MK versus UW	MK	Porcine	[12]
MK versus UW	MK	Human	[41] (in discussion)
MK versus ET-K	MK	Rat	[12]
MK versus M-HTK	MK	Porcine	[43]
MK versus HNC	MK	Human	[41]

UW: University of Wisconsin solution; MK: modified ET-Kyoto solution (ET-Kyoto solution with ulinastatin); ET-K: ET-Kyoto solution; M-HTK: modified histidine-tryptophan-ketoglutarate solution (HTK solution with ulinastatin); HNC: Celsior solution with HES and nafamostat mesilate.

5. Trypsin Inhibitors in Preservation Solution

Trypsin from pancreatic acinar cells destroys islets. Previous study has shown that trypsin inhibition by Pefabloc during human pancreas digestion improves islet yield and reduces the fraction of embedded (trapped) islets [64], thus suggesting that trypsin may degrade the ductules and thus reduce the delivery of collagenase solution to tissue around the islets. We previously reported that pancreas

TABLE 3: Trypsin inhibitors in ET-Kyoto solution.

Trypsin inhibitors	Trypsin inhibition	Isle yield versus ulinastatin	Viability versus ulinastatin	SI* versus ulinastatin
Ulinastatin	++	—	—	—
Pefabloc	+	Lower	n.s.	Lower
Gabexate mesilate	+++	n.s.	Lower	Lower
Nafamostat mesilate	++++	n.s.	Lower	n.s.

* Stimulation index; n.s.: not significant.

preservation using MK solution including ulinastatin, which eliminated trypsin activity during pancreas preservation, was superior to that using ET-Kyoto solution without the trypsin inhibitor in a rat model [12]. Furthermore, the advantages of MK solution are its trypsin inhibition and less collagenase inhibition in human and porcine islet isolation [12, 47]. Therefore, we compared ulinastatin with other trypsin inhibitors, including Pefabloc, gabexate mesilate, and nafamostat mesilate, in preservation solution for porcine islet isolation [62, 63]. Trypsin inhibition is greater in ET-Kyoto with gabexate mesilate (GK) solution and ET-Kyoto with nafamostat mesilate (NK) solution than in MK solution. The islet yield before purification was higher in the MK group than in the ET-Kyoto with Pefabloc (PK) group. Viability was higher for the MK group than for either the GK group or the NK group. The stimulation index was higher for the MK group than for either the PK group or the GK group. These data suggest that MK solution was synthetically superior to the PK, GK, or NK solutions, although trypsin inhibition is greater in GK and NK solutions than in MK solution (Table 3) [62, 63], possibly due to differences of inhibitory effects of cytokines. Ulinastatin has been shown to inhibit not only trypsin activity but also the release of neutrophil elastase. It also downregulates transcription of TNF mRNA, the activation of endothelial cells, and the expression of ICAM-1 induced by endotoxin *in vitro* [65–67]. The administration of ulinastatin has been shown to decrease ischemia-reperfusion injury [68] or attenuate the elevation in the concentrations of inflammatory cytokines and C-reactive protein, a marker of inflammation [69], in the transplanted small intestine.

Recently, the importance of tryptic-like activity (TLA) obtained from *Clostridium histolyticum* in collagenase NB1 with neutral protease for efficient islet isolation was demonstrated [70]. Enhancing TLA resulted in a significant reduction of recirculation time and incrementally increased human islet yield. The clostridial TLA and pancreatic trypsin seemed to be different in their specificity toward islet and nonislet pancreatic tissue because no detrimental effect on islet viability and integrity was detected on clostridial TLA. If trypsin inhibitors inhibit clostridial TLA as well as pancreatic trypsin, then they may inhibit pancreas digestion. This may explain the synthetic superiority of MK solution to PK, GK, or NK solutions, although trypsin inhibition is greater in GK and NK solutions than in MK solution (Figure 1(c)).

6. Two-Layer Method

The two-layer preservation method (TLM), which uses the concept of normobaric oxygenation comprising a cold organ preservation solution (UW solution) with a perfluorochemical (PFC) oxygen carrier solution, with the pancreas being suspended between the two immiscible layers, has been utilized for many clinical trials of islet transplantation [71–74]. However, two recent large-scale studies showed no beneficial effect of TLM, compared with UW storage, on human islet isolation and transplantation [75, 76]. We reevaluated the effect of TLM using three different groups: group 1: UW simple storage; group 2: TLM performed by multiorgan procurement teams (not specialists in islet isolation); group 3: TLM performed by specialists in islet isolation. There were no significant differences between group 1 and 2, whereas islet yields were significantly higher for group 3 compared with either groups 1 or 2. Our data suggest that performance of TLM by experts could improve the outcome of islet isolation and transplantation [71].

On the other hand, Papas et al. showed that the oxygen penetration depth is about 1 mm, suggesting that pancreas oxygenation is limited during preservation with the TLM [77]. In other words, their data suggest that the percentage of pancreas oxygenation by TLM depends on its thickness and the trimming of the pancreas before preservation by TLM is thus considered to be important for pancreas oxygenation.

7. Conclusion

ET-Kyoto with ulinastatin was the best combination for pancreas preservation in our studies. Since one of the advantages of MK solution is less collagenase inhibition in islet isolation, it is also suitable for ductal injection. Based on these data, we now use the *in situ* regional cooling system for DCD pancreata, the ductal injection of preservation solution, and pancreas preservation by MK solution during clinical islet isolation/transplantation. The *in situ* regional cooling system to DCD pancreata, ductal injection, and preservation by MK solution is therefore considered to be useful improvement that may help to increase organ utilization and thereby achieve good outcomes after islet transplantation.

Conflict of Interest

The author of this manuscript has no conflict of interests.

References

- [1] A. M. J. Shapiro, J. R. T. Lakey, E. A. Ryan et al., "Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen," *The New England Journal of Medicine*, vol. 343, no. 4, pp. 230–238, 2000.
- [2] E. A. Ryan, J. R. T. Lakey, R. V. Rajotte et al., "Clinical outcomes and insulin secretion after islet transplantation with the edmonton protocol," *Diabetes*, vol. 50, no. 4, pp. 710–719, 2001.
- [3] J. F. Markmann, S. Deng, N. M. Desai et al., "The use of non-heart-beating donors for isolated pancreatic islet transplantation," *Transplantation*, vol. 75, no. 9, pp. 1423–1429, 2003.
- [4] H. A. Clayton, S. M. Swift, J. M. Turner, R. F. L. James, and P. R. F. Bell, "Non-heart-beating organ donors: a potential source of islets for transplantation," *Transplantation*, vol. 69, no. 10, pp. 2094–2098, 2000.
- [5] H. Noguchi, Y. Yamada, T. Okitsu et al., "Secretory unit of islet in transplantation (SUIT) and engrafted islet rate (EIR) indexes are useful for evaluating single islet transplantation," *Cell Transplantation*, vol. 17, no. 1-2, pp. 121–128, 2008.
- [6] H. Noguchi, Y. Iwanaga, T. Okitsu, H. Nagata, Y. Yonekawa, and S. Matsumoto, "Evaluation of islet transplantation from non-heart beating donors," *American Journal of Transplantation*, vol. 6, no. 10, pp. 2476–2482, 2006.
- [7] B. J. Hering, R. Kandaswamy, J. D. Ansite et al., "Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes," *Journal of the American Medical Association*, vol. 293, no. 7, pp. 830–835, 2005.
- [8] B. J. Hering, R. Kandaswamy, J. V. Harmon et al., "Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody," *American Journal of Transplantation*, vol. 4, no. 3, pp. 390–401, 2004.
- [9] B. J. Hering, I. Matsumoto, T. Sawada et al., "Impact of two-layer pancreas preservation on islet isolation and transplantation," *Transplantation*, vol. 74, no. 12, pp. 1813–1816, 2002.
- [10] S. Matsumoto, M. Takita, D. Chaussabel et al., "Improving efficacy of clinical islet transplantation with iodixanol based islet purification, thymoglobulin induction and blockage of IL-1-beta and TNF-alpha," *Cell Transplantation*. In press.
- [11] S. Matsumoto, T. Okitsu, Y. Iwanaga et al., "Insulin independence after living-donor distal pancreatectomy and islet allotransplantation," *Lancet*, vol. 365, no. 9471, pp. 1642–1644, 2005.
- [12] H. Noguchi, M. Ueda, Y. Nakai et al., "Modified two-layer preservation method (M-Kyoto/PFC) improves islet yields in islet isolation," *American Journal of Transplantation*, vol. 6, no. 3, pp. 496–504, 2006.
- [13] H. Noguchi, M. F. Levy, N. Kobayashi, and S. Matsumoto, "Pancreas preservation by the two-layer method: does it have a beneficial effect compared with simple preservation in University of Wisconsin solution?" *Cell Transplantation*, vol. 18, no. 5-6, pp. 497–503, 2009.
- [14] H. Noguchi, "Pancreatic islet transplantation," *World Journal of Gastrointestinal Surgery*, vol. 1, no. 1, pp. 16–20, 2009.
- [15] H. Noguchi, T. Ikemoto, B. Naziruddin et al., "Iodixanol-controlled density gradient during islet purification improves recovery rate in human islet isolation," *Transplantation*, vol. 87, no. 11, pp. 1629–1635, 2009.
- [16] H. Noguchi, S. Matsumoto, N. Onaca et al., "Ductal injection of JNK inhibitors before pancreas preservation prevents islet apoptosis and improves islet graft function," *Human Gene Therapy*, vol. 20, no. 1, pp. 73–85, 2009.
- [17] H. Noguchi, B. Naziruddin, M. Shimoda et al., "A combined continuous density/osmolality gradient for supplemental purification of human islets," *Cell Medicine*. In press.
- [18] H. Noguchi, Y. Nakai, S. Matsumoto et al., "Cell permeable peptide of JNK inhibitor prevents islet apoptosis immediately after isolation and improves islet graft function," *American Journal of Transplantation*, vol. 5, no. 8, pp. 1848–1855, 2005.
- [19] H. Noguchi, Y. Nakai, M. Ueda et al., "Activation of c-Jun NH2-terminal kinase (JNK) pathway during islet transplantation and prevention of islet graft loss by intraportal injection

- of JNK inhibitor," *Diabetologia*, vol. 50, no. 3, pp. 612–619, 2007.
- [20] H. Noguchi, B. Naziruddin, A. Jackson et al., "Low-temperature preservation of isolated islets is superior to conventional islet culture before islet transplantation," *Transplantation*, vol. 89, no. 1, pp. 47–54, 2010.
- [21] H. Noguchi, "Cell-permeable peptide inhibitor of c-Jun NH2-terminal kinase for the treatment of diabetes," *Current Bioactive Compounds*, vol. 6, no. 1, pp. 23–30, 2010.
- [22] H. Noguchi, M. Matsushita, T. Okitsu et al., "A new cell-permeable peptide allows successful allogeneic islet transplantation in mice," *Nature Medicine*, vol. 10, no. 3, pp. 305–309, 2004.
- [23] J. R. T. Lakey, G. L. Warnock, R. V. Rajotte et al., "Variables in organ donors that affect the recovery of human islets of Langerhans," *Transplantation*, vol. 61, no. 7, pp. 1047–1053, 1996.
- [24] D. Brandhorst, B. J. Hering, H. Brandhorst, K. Federlin, and R. G. Bretzel, "Influence of donor data and organ procurement on human islet isolation," *Transplantation Proceedings*, vol. 26, no. 2, pp. 592–593, 1994.
- [25] P. Fiedor, E. R. Goodman, R. S. Sung, J. Czerwiński, W. Rowiński, and M. A. Hardy, "The effect of clinical and biochemical donor parameters on pancreatic islet isolation yield from cadaveric organ donors," *Annals of Transplantation*, vol. 1, no. 1, pp. 59–62, 1996.
- [26] Y. Zeng, M. A. Torre, T. Karrison, and J. R. Thistlethwaite, "The correlation between donor characteristics and the success of human islet isolation," *Transplantation*, vol. 57, no. 6, pp. 954–958, 1994.
- [27] J. F. Markmann, S. Deng, N. M. Desai et al., "The use of non-heart-beating donors for isolated pancreatic islet transplantation," *Transplantation*, vol. 75, no. 9, pp. 1423–1429, 2003.
- [28] H. A. Clayton, S. M. Swift, J. M. Turner, R. F. L. James, and P. R. F. Bell, "Non-heart-beating organ donors: a potential source of islets for transplantation," *Transplantation*, vol. 69, no. 10, pp. 2094–2098, 2000.
- [29] H. Nagata, S. Matsumoto, T. Okitsu et al., "Procurement of the human pancreas for pancreatic islet transplantation from marginal cadaver donors," *Transplantation*, vol. 82, no. 3, pp. 327–331, 2006.
- [30] M. Kato, K. Mizutani, R. Hattori et al., "In situ renal cooling for kidney transplantation from non-heart-beating donors," *Transplantation Proceedings*, vol. 32, no. 7, pp. 1608–1610, 2000.
- [31] Y. Yonekawa, S. Matsumoto, T. Okitsu et al., "Effective islet isolation method with extremely high islet yields from adult pigs," *Cell Transplantation*, vol. 14, no. 10, pp. 757–762, 2005.
- [32] J. J. O'Neil, J. P. Stegemann, D. T. Nicholson, K. A. Gagnon, B. A. Solomon, and C. J. P. Mullon, "The isolation and function of porcine islets from market weight pigs," *Cell Transplantation*, vol. 10, no. 3, pp. 235–246, 2001.
- [33] C. Ricordi, P. E. Lacy, E. H. Finke, B. J. Olack, and D. W. Scharp, "Automated method for isolation of human pancreatic islets," *Diabetes*, vol. 37, no. 4, pp. 413–420, 1988.
- [34] S. R. Munn, D. B. Kaufman, M. J. Field, A. B. Viste, and D. E. R. Sutherland, "Cold-storage preservation of the canine and rat pancreas prior to islet isolation," *Transplantation*, vol. 47, no. 1, pp. 28–31, 1989.
- [35] H. Noguchi, M. Ueda, S. Hayashi et al., "Ductal injection of preservation solution increases islet yields in islet isolation and improves islet graft function," *Cell Transplantation*, vol. 17, no. 1-2, pp. 69–81, 2008.
- [36] T. Sawada, I. Matsumoto, M. Nakano, N. Kirchhof, D. E. R. Sutherland, and B. J. Hering, "Improved islet yield and function with ductal injection of University of Wisconsin solution before pancreas preservation," *Transplantation*, vol. 5, no. 12, pp. 1965–1969, 2003.
- [37] T. Tojimbara, W. N. Wicomb, R. Garcia-Kennedy et al., "Liver transplantation from non-heart beating donors in rats: influence of viscosity and temperature of initial flushing solutions on graft function," *Liver Transplantation and Surgery*, vol. 3, no. 1, pp. 39–45, 1997.
- [38] G. S. Robertson, D. Chadwick, S. Thirdborough et al., "Human islet isolation—a prospective randomized comparison of pancreatic vascular perfusion with hyperosmolar citrate or University of Wisconsin solution," *Transplantation*, vol. 56, no. 3, pp. 550–553, 1993.
- [39] H. H. Contractor, P. R. V. Johnson, D. R. Chadwick, G. S. M. Robertson, and N. J. M. London, "The effect of UW solution and its components on the collagenase digestion of human and porcine pancreas," *Cell Transplantation*, vol. 4, no. 6, pp. 615–619, 1995.
- [40] M. Omasa, S. Hasegawa, T. Bando et al., "Application of ET-Kyoto solution in clinical lung transplantation," *Annals of Thoracic Surgery*, vol. 77, no. 1, pp. 338–339, 2004.
- [41] F. Chen, T. Fukuse, S. Hasegawa et al., "Effective application of ET-Kyoto solution for clinical lung transplantation," *Transplantation Proceedings*, vol. 36, no. 9, pp. 2812–2815, 2004.
- [42] S. F. Wu, Y. Suzuki, A. K. Kitahara, H. Wada, and Y. Nishimura, "Skin flap storage with intracellular and extracellular solutions containing trehalose," *Annals of Plastic Surgery*, vol. 43, no. 3, pp. 289–294, 1999.
- [43] R. Troisi, D. Meester, C. Van Den Broecke et al., "Functional and structural integrity of porcine pancreatic grafts subjected to a period of warm ischemia and cold preservation with histidine-tryptophan-ketoglutarate (custodiol) or University of Wisconsin solution," *Transplantation*, vol. 75, no. 11, pp. 1793–1799, 2003.
- [44] F. O. Belzer and J. H. Southard, "Organ preservation and transplantation," *Progress in Clinical and Biological Research*, vol. 224, pp. 291–303, 1986.
- [45] D. R. Chadwick, G. S. M. Robertson, H. H. Contractor et al., "Storage of pancreatic digest before islet purification: the influence of colloids and the sodium to potassium ratio in University of Wisconsin-based preservation solutions," *Transplantation*, vol. 58, no. 1, pp. 99–104, 1994.
- [46] S. Fujimoto, E. R. I. Mukai, Y. Hamamoto et al., "Prior exposure to high glucose augments depolarization-induced insulin release by mitigating the decline of ATP level in rat islets," *Endocrinology*, vol. 143, no. 1, pp. 213–221, 2002.
- [47] H. Noguchi, B. Naziruddin, N. Onaca et al., "Comparison of modified Celsior solution and M-Kyoto solution for pancreas preservation in human islet isolation," *Cell Transplantation*, vol. 19, no. 6-7, pp. 751–758, 2010.
- [48] H. J. Bretschneider, "Myocardial protection," *The Thoracic and Cardiovascular Surgeon*, vol. 28, no. 5, pp. 295–302, 1980.
- [49] J. Erhard, R. Lange, R. Scherer et al., "Comparison of histidine-tryptophan-ketoglutarate (HTK) solution versus University of Wisconsin (UW) solution for organ preservation in human liver transplantation. A prospective, randomized study," *Transplant International*, vol. 7, no. 3, pp. 177–181, 1994.
- [50] U. J. Hesse, R. Troisi, B. Jacobs et al., "Cold preservation of the porcine pancreas with histidine-tryptophan-ketoglutarate solution," *Transplantation*, vol. 66, no. 9, pp. 1137–1141, 1998.
- [51] U. Leonhardt, A. Tytko, B. Exner et al., "The effect of different solutions for organ preservation of immediate postischemic

- pancreatic function in vitro," *Transplantation*, vol. 55, no. 1, pp. 11–14, 1993.
- [52] S. Poddar, S. Malek, B. Eghtesad et al., "Initial experience using histidine-tryptophan-ketoglutarate solution in clinical pancreas transplantation," *Clinical Transplantation*, vol. 18, no. 6, pp. 661–665, 2004.
- [53] M. J. Englesbe, A. Moyer, D. Y. Kim et al., "Early pancreas transplant outcomes with histidine-tryptophan-ketoglutarate preservation: a multicenter study," *Transplantation*, vol. 82, no. 1, pp. 136–139, 2006.
- [54] J. A. Fridell, A. Agarwal, M. L. Milgrom, W. C. Goggins, P. Murdock, and M. D. Pescovitz, "Comparison of histidine-tryptophan-ketoglutarate solution and University of Wisconsin solution for organ preservation in clinical pancreas transplantation," *Transplantation*, vol. 77, no. 8, pp. 1304–1306, 2004.
- [55] H. Noguchi, M. Ueda, S. Hayashi et al., "Comparison of M-Kyoto solution and histidine-tryptophan-ketoglutarate solution with a trypsin inhibitor for pancreas preservation in islet transplantation," *Transplantation*, vol. 84, no. 5, pp. 655–658, 2007.
- [56] J. Caballero-Corbalán, H. Brandhorst, H. Malm et al., "Using HTK for prolonged pancreas preservation prior to human islet isolation," *The Journal of Surgical Research*. In press.
- [57] P. Menasché, J. L. Termignon, F. Pradier et al., "Experimental evaluation Celsior, a new heart preservation solution," *European Journal of Cardio-Thoracic Surgery*, vol. 8, no. 4, pp. 207–213, 1994.
- [58] R. F. Roberts, G. P. Nishanian, J. N. Carey, Y. Sakamaki, V. A. Starnes, and M. L. Barr, "A comparison of the new preservation solution Celsior to Euro-Collins and University of Wisconsin solutions in lung reperfusion injury," *Transplantation*, vol. 67, no. 1, pp. 152–155, 1999.
- [59] A. Cavallari, U. Cillo, B. Nardo et al., "A multicenter pilot-prospective study comparing Celsior and University of Wisconsin preservingsolutions for use in liver transplantation," *Liver Transplantation*, vol. 9, no. 8, pp. 814–821, 2003.
- [60] A. Faenza, F. Catena, B. Nardo et al., "Kidney preservation with university of Wisconsin and Celsior solution: a prospective multicenter randomized study," *Transplantation*, vol. 72, no. 7, pp. 1274–1277, 2001.
- [61] T. Hubert, V. Gmyr, L. Arnalsteen et al., "Influence of preservation solution on human islet isolation outcome," *Transplantation*, vol. 83, no. 3, pp. 270–276, 2007.
- [62] H. Noguchi, M. Ueda, S. Hayashi et al., "Comparison of trypsin inhibitors in preservation solution for islet isolation," *Cell Transplantation*, vol. 18, no. 5-6, pp. 541–547, 2009.
- [63] H. Noguchi, B. Naziruddin, A. Jackson et al., "Comparison of ulinastatin, gabexatemesilate, and nafamostatmesilate in preservation solution for islet isolation," *Cell Transplantation*. In press.
- [64] J. R. T. Lakey, L. M. H. Helms, T. Kin et al., "Serine-protease inhibition during islet isolation increases islet yield from human pancreases with prolonged ischemia," *Transplantation*, vol. 72, no. 4, pp. 565–570, 2001.
- [65] S. Aosasa, S. Ono, H. Mochizuki, H. Tsujimoto, C. Ueno, and A. Matsumoto, "Mechanism of the inhibitory effect of protease inhibitor on tumor necrosis factor α production of monocytes," *Shock*, vol. 15, no. 2, pp. 101–105, 2001.
- [66] S. Aosasa, S. Ono, S. Seki et al., "Inhibitory effect of protease inhibitor on endothelial cell activation," *Journal of Surgical Research*, vol. 80, no. 2, pp. 182–187, 1998.
- [67] K. Nakatani, S. Takeshita, H. Tsujimoto, Y. Kawamura, and I. Sekine, "Inhibitory effect of serine protease inhibitors on neutrophil-mediated endothelial cell injury," *Journal of Leukocyte Biology*, vol. 69, no. 2, pp. 241–247, 2001.
- [68] J. L. Sun, X. Q. Zhang, Z. G. Yu et al., "Effects of ulinastatin on postoperative systemic inflammatory response of recipients of rat small bowel transplantation," *Transplantation Proceedings*, vol. 38, no. 6, pp. 1803–1804, 2006.
- [69] X. Q. Zhang, J. L. Sun, Y. J. Chen, R. Ma, X. H. Fan, and J. Z. Sun, "Amelioration of ischemia-reperfusion injury of transplanted small intestine by ulinastatin: effects on accumulation and adhesion of neutrophil," *Transplantation Proceedings*, vol. 37, no. 10, pp. 4464–4466, 2005.
- [70] H. Brandhorst, A. Friberg, H. H. Andersson et al., "The importance of tryptic-like activity in purified enzyme blends for efficient islet isolation," *Transplantation*, vol. 87, no. 3, pp. 370–375, 2009.
- [71] H. Noguchi, M. F. Levy, N. Kobayashi, and S. Matsumoto, "Pancreas preservation by the two-layer method: does it have a beneficial effect compared with simple preservation in University of Wisconsin solution?" *Cell Transplantation*, vol. 18, no. 5-6, pp. 497–503, 2009.
- [72] T. Tsujimura, Y. Kuroda, T. Kin et al., "Human islet transplantation from pancreases with prolonged cold ischemia using additional preservation by the two-layer (UW solution/perfluorochemical) cold-storage method," *Transplantation*, vol. 74, no. 12, pp. 1687–1691, 2002.
- [73] C. Ricordi, C. Fraker, J. Szust et al., "Improved human islet isolation outcome from marginal donors following addition of oxygenated perfluorocarbon to the cold-storage solution," *Transplantation*, vol. 75, no. 9, pp. 1524–1527, 2003.
- [74] B. J. Hering, R. Kandaswamy, J. V. Harmon et al., "Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody," *American Journal of Transplantation*, vol. 4, no. 3, pp. 390–401, 2004.
- [75] J. Caballero-Corbalán, T. Eich, T. Lundgren et al., "No beneficial effect of two-layer storage compared with UW-storage on human islet isolation and transplantation," *Transplantation*, vol. 84, no. 7, pp. 864–869, 2007.
- [76] T. Kin, M. Mirbolooki, P. Salehi et al., "Islet isolation and transplantation outcomes of pancreas preserved with University of Wisconsin solution versus two-layer method using preoxygenated perfluorocarbon," *Transplantation*, vol. 82, no. 10, pp. 1286–1290, 2006.
- [77] K. K. Papas, B. J. Hering, L. Gunther, M. J. Rappel, C. K. Colton, and E. S. Avgoustiniatos, "Pancreas oxygenation is limited during preservation with the two-layer method," *Transplantation Proceedings*, vol. 37, no. 8, pp. 3501–3504, 2005.

Research Article

Human Liver Cells Expressing Albumin and Mesenchymal Characteristics Give Rise to Insulin-Producing Cells

Irit Meivar-Levy,¹ Tamar Sapir,² Dana Berneman,^{1,3} Tal Weissbach,⁴ Sylvie Polak-Charcon,⁵ Philippe Ravassard,⁶ Andreas G. Tzakis,⁷ Eytan Mor,⁸ Camillo Ricordi,² and Sarah Ferber^{1,3}

¹Sheba Regenerative Medicine, Stem cells and Tissue engineering Center, Sheba Medical Center, Tel-Hashomer 52621, Israel

²Diabetes Research Institute, University of Miami Leonard M. Miller School of Medicine, 1450 NW 10th Avenue, Miami, FL 33136, USA

³Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel-Aviv University, 69978 Tel-Aviv, Israel

⁴Obstetrics and Gynecology Department, Sapir Medical Center 44281 Kfar Saba, Israel

⁵The Institute for Pathology, Sheba Medical Center, 52621 Tel-Hashomer, Israel

⁶Biotechnology and Biotherapy Group, Centre de Recherche, Institut du Cerveau et de la Moelle CNRS UMR7225, INSERM UMRS795, Université Pierre et Marie Curie, 75005 Paris, France

⁷The Miami Transplant Institute, University of Miami Leonard M. Miller School of Medicine, 1450 NW 10th Avenue, Miami, FL 33136, USA

⁸Rabin Medical Center, Beilinson Campus, 49100 Petah-Tiqva, Israel

Correspondence should be addressed to Sarah Ferber, sferber@sheba.health.gov.il

Received 28 April 2011; Accepted 5 June 2011

Academic Editor: Thierry Berney

Copyright © 2011 Irit Meivar-Levy et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Activation of the pancreatic lineage in the liver has been suggested as a potential autologous cell replacement therapy for diabetic patients. Transcription factors-induced liver-to-pancreas reprogramming has been demonstrated in numerous species both *in vivo* and *in vitro*. However, human-derived liver cells capable of acquiring the alternate pancreatic repertoire have never been characterized. It is yet unknown whether hepatic-like stem cells or rather adult liver cells give rise to insulin-producing cells. Using an *in vitro* experimental system, we demonstrate that proliferating adherent human liver cells acquire mesenchymal-like characteristics and a considerable level of cellular plasticity. However, using a lineage-tracing approach, we demonstrate that insulin-producing cells are primarily generated in cells enriched for adult hepatic markers that coexpress both albumin and mesenchymal markers. Taken together, our data suggest that adult human hepatic tissue retains a substantial level of developmental plasticity, which could be exploited in regenerative medicine approaches.

1. Introduction

A cure for type 1 diabetes mellitus depends on replenishing functional insulin-producing cells. However, the limited supply of pancreatic islets from cadaver donors and the need for life-long immune suppression makes pancreas or pancreatic islet allotransplantation impractical for the vast majority of patients. This hurdle has led to a search for new alternate sources of insulin-producing cells or tissues [1–3]. A challenging approach to generating surrogate β -cells for cell replacement therapy in diabetes is the direct reprogramming of liver cells into insulin-producing cells.

Liver and pancreatic cells share a common developmental origin, making liver cells good candidates for manipulation into β -like cells [4]. Both tissues may share a common population of progenitor cells [5]. For example, cells with properties virtually identical to those of hepatic oval cells can also emerge in the pancreas, especially after the ablation of acinar cells [6]. Upon transplantation, these pancreas-derived oval cells can differentiate into functional hepatocytes and bile ducts [7]. The reciprocal conversion of rodent [8–13] and human [14–18] liver cells into pancreatic endocrine cells by transdifferentiation or direct cellular reprogramming has also been described. Liver cells have

been induced to differentiate into insulin-producing cells by ectopic expression of pancreatic transcription factors, the best studied of which is PDX-1, a key regulator of pancreatic development and insulin expression in adult pancreatic beta cells [8, 10, 14, 16–19]. However, whether transcription factor-induced reprogramming primarily occurs in “stem-like” pluripotent cells or rather adult cells can directly give rise to committed cells of alternate lineages is questionable and presently analyzed.

Adult liver contains several populations of cells, including hepatocytes, cholangiocytes, endothelial stellate cells, and bone marrow- (BM-) derived cells [20–22]. Activation of the pancreatic lineage in mice *in vivo* has been reported to occur in several areas of the intact organ [8, 11, 23, 24]. PDX-1-induced insulin production in mouse livers *in vivo* appears to occur mainly in the parenchyma of the liver around the central veins [8, 11, 25]. On the other hand, NEUROD1 and betacellulin-induced insulin production occurs mainly in cells close to the hepatic capsule [24]. NGN-3 and betacellulin induce the transdetermination of parenchymal hepatocytes and hepatic progenitor cells and possibly endoderm-derived oval cells in periportal areas of the liver [26].

The aim of the present study was to characterize the human-derived liver cells capable of giving rise to insulin-producing cells and determine whether they originate from mature or hepatic progenitor cells. The definite characteristics of hepatic progenitor cells which populate the adult human organ is controversial; however, there is a wide agreement that all these populations express the epithelial marker, EpCAM [27–29]. We generated *in vitro* primary cultures of liver cells derived from different human donors that already demonstrated a capacity to reprogram along the endocrine pancreatic and β -cell-like lineages by ectopic expression of pancreatic transcription factors [14–16, 30]. The origin of the induced insulin-positive cells in proliferating cultures of adult human liver cells is not clear, because the original liver cell morphology is altered and the cells undergo massive dedifferentiation, which is further augmented by ectopic PDX-1 expression and the reprogramming process itself [30]. Here, we characterize the cells in the adherent, proliferating cultures derived from adult human liver and demonstrate their mesenchymal-like characteristics. Using a genetic cell lineage tracing for albumin, we demonstrate that the cells coexpress both mesenchymal and adult hepatic markers, but none of the cells express EpCAM. The mesenchymal-like cells that originate from cells expressing albumin give rise to insulin expression upon ectopic PDX-1 expression.

2. Material and Methods

2.1. Human Liver Cells. Adult human liver tissues were obtained from 3 different liver transplantation surgeries from 4–10-years-old children and 8 individuals over forty years old. Liver tissues were used with approval from the Committee on Clinical Investigations (institutional review board).

Isolation of human liver cells was performed as previously described [16, 31]. Briefly, the cells were digested by

0.03% Collagenase type I (Worthington Biochemical Corp., NJ) and cultured in Dulbecco's minimal essential medium (1 gr/L glucose) supplemented with 10% FCS, 100 U/mL penicillin, 100 μ g/mL streptomycin and 250 ng/mL amphotericin B (Biological Industries, Israel). The medium was changed daily during the first three days in order to remove nonadherent cells. Ninety percent confluent cultures were split using trypsin-EDTA. The cells were kept at 37°C in a humidified atmosphere of 5% CO₂ and 95% air.

2.2. Lentivirus Vector Construction and Virus Production. The pTrip albumin promoter (410) nlsCRE DeltaU3 (ALB-Cre) vector was generated by removing with *Bam*HI and *Xho*I of the enhanced green fluorescent protein (eGFP) coding region from the pTrip ALB eGFP DeltaU3 vector, which contains a fragment of the rat albumin promoter from –423 to –23 relative to the transcription start site. The resulting linearized plasmid was blunt-ended with DNA polymerase I Klenow fragment. The reading frame A Gateway cassette (Gateway Conversion kit; Invitrogen) was next ligated to the blunt-ended vector according to the manufacturer's instructions, generating a pTrip ALB rfa-Gateway DeltaU3 destination vector. The nlsCRE fragment was amplified by PCR from a plasmid [32] provided by Guilan Vodjdani (Hospital de la Pitie', Salpetriere, Paris) using the forward primer 5' CACCAGATCTATGCCCAAGAAGA.

AGAGG-3' and the reverse primer 5'-CTCGAGCTA-ATCGCCATCTTC-3', and the resulting PCR product was cloned into the pENTR/D/TOPO plasmid (Invitrogen) to generate an nls-CRE entry clone. Both destination vector and entry clone were used for *in vitro* recombination using the LR clonase II system (Invitrogen) according to the manufacturer's instructions. The reporter vector was constructed as previously reported [33].

Virus particles were produced in 293T cells after pCMVdr8.91 and pMD2.G vectors cotransfection. The culture medium was harvested 36–48 h later.

2.3. Viral Infection. Lentiviruses infection was performed 24 hours after plating; liver cells were washed with PBS and infected with a 1 : 1 mixture of the two viruses at multiplicity of infection (MOI) 3 : 1 in growth media containing 8 ng/mL polybrene overnight. The medium was then replaced with culture medium, and the cells were refed twice a week and split 1 : 3 once a week. The percentiles of eGFP and DsRed2 positive cells were analyzed using a Beckman Coulter FC500 flow cytometer or FACS Calibur, using the CellQuest program.

Adenoviral infection of *Ad-CMV-PDX-1* (1000 MOI) was performed as previously reported [14, 16, 30].

2.4. Animal Studies. All animals were maintained and animal experiments were carried out under the supervision and guidelines of the Sheba Medical center Institutional Animal Welfare Committee (177/2002).

Cells at passage 4, were harvested, washed twice with sterile PBS, counted, and resuspended in Matrigel (BD Biosciences). Six-week-old female athymic nude mice were

injected subcutaneously in both flanks with human liver cells at density of 1×10^6 viable cells/100 μ L as previously described [34]. Five mice were used in each group. Tumor size was measured with a linear caliper for up to 17 weeks.

2.5. Flow Cytometry. Liver-derived cells were harvested and washed with flow cytometry buffer consisting of 1% BSA and 0.1% sodium azide (Sigma, St. Louis, Mo, USA) in phosphate buffered saline (Invitrogen, Carlsbad, Calif, USA). For the cell surface antigen detection, approximately 10^5 cells labeled with conjugated monoclonal antibodies. Intracellular staining was performed using Intracellular Staining Flow Assay Kit (Imgenex, San Diego, Calif, USA) following manufacturer's instruction. Control samples included unstained cells, isotype antibody stained cells, and single fluorochrome-stained cells. The antibodies used in this study are listed in supplemental material data 1.

The cells were analyzed using a Beckman Coulter FC500 flow cytometer or FACS Calibur, using the CellQuest program.

2.6. Cell Sorting. Three weeks after lentiviruses infection the labeled liver-derived cells were sorted using a fluorescence-activated cell sorter (FACS) (Aria cell sorter; Becton Dickinson, San Jose, Calif, USA) with a fluorescein isothiocyanate filter (530/30 nm) for eGFP and a Pe-Texas Red filter (610/20 nm) for DsRed2.

2.7. In Vitro Adipogenic and Osteogenic Differentiation. Following manufacturer's instructions (Human Mesenchymal Stem Cell Functional Identification Kit, R&D Systems, Minneapolis, Minn, USA), human liver-derived cells at passage 4 were plated on cover slips at 2,000 cells/cm² in 6-well tissue culture-treated plates in the presence of the adipogenic or osteogenic supplements provided by the company. The appropriate supplemented medium was changed twice per week. After 14 days in culture, the adipogenic culture formed adipogenic-like vacuoles. The plates were fixed with 4% paraformaldehyde for 20 minutes and stained with Oil Red O (Sigma). The osteogenic differentiation cultures were incubated for 21 days and fixed and stained with 1% Alizarin Red solution pH 4.1 (Sigma). The calcium deposits were stained orange-red. Slides were imaged under a Leica DMLB microscope using the Leica Application Suite version 2.7.1 R1 software.

2.8. RNA Isolation, RT and RT-PCR Reactions. Total RNA was isolated, cDNA was prepared and amplified as described previously [8, 16]. Quantitative real-time RT-PCR was performed using ABI StepOnePlus (Applied Biosystems, Calif, USA) as described previously [14, 16, 30]. The primer pairs and annealing temperatures listed in supplemental material data 2.

2.9. Immunofluorescence. Human liver cells treated were plated on glass cover slides in six-well culture plates. Forty-eight hours later, the cells were fixed and stained as described previously [16]. The antibodies used in this study are listed in supplemental material data 1.

The slides were analyzed using a fluorescent microscope (Provis, Olympus).

2.10. Statistical Analyses. Statistical analyses were performed using two-sample Students *t*-test assuming unequal variances.

3. Results

3.1. Characterization of Human Liver-Derived Cells Cultured In Vitro. Adult human liver cells can be propagated *in vitro* for roughly 20 passages; after an initial 2-week lag, the cells proliferate at a constant rate (Figure 1(a)) [14–17]. In addition to proliferation, a gradual and partial decrease in mature hepatic characteristics is observed [30]. A comparison of the gene expression profiles of primary cultures of adult human liver cells (passages 2–4), and the original intact tissues revealed changes in the repertoire of expressed genes (Figures 1(b) and 1(c)). Human liver-derived cells in culture undergo dedifferentiation, manifested as decreased expression of numerous adult hepatic markers and increased expression of immature and endodermal markers (Figure 1(b)) [30]. Previously, it was reported that hepatic dedifferentiation and downregulation of mature hepatic markers occurs rapidly, within 24 hours in culture [35]. Our data support that as the reduction was detected at any time point analyzed (P0–25 in culture). Despite the massive downregulation of adult hepatic markers, $88 \pm 6\%$ of the liver cells in culture maintains albumin expression and production, as demonstrated by flow cytometry and immunofluorescence (Figures 2(a) and 2(b)). Approximately 60% of the cells in culture were positive for another adult hepatic marker α -anti-trypsin (AAT) inhibitor (Figure 2(a)) though at lower levels than in the intact organ [30]. Twenty percent of liver cells in culture express the hepatic fetal marker alpha-fetoprotein (AFP), but none express the hepatic progenitor marker EpCAM or duct cell markers CA19–9 or CK19 [28, 36, 37]. Taken together, these results suggest that the proliferating cells in culture may not represent populations of hepatic stem cells.

Liver cells in culture expressed lower levels of the epithelial marker E-CAD and higher levels of N-CAD compared to intact liver tissues (Figures 1(c) and 2(a)). Such a switch in cadherin expression usually characterizes an epithelial to mesenchymal transition (EMT) process, which also occurs to pancreatic islet cells in culture [38]. In addition to the switch in cadherin expression levels, expression of the transcription factors Snail and Slug was activated, which further strengthens the notion that proliferating human liver cells in culture may undergo an EMT process (Figure 1(c)).

To further uncover the nature and properties of liver-derived cells in *in vitro* culture, we analyzed the expression of markers that characterize mesenchymal cells. Cellular characterization of the cells in increasing passages indicated that most liver-derived cells in culture express several mesenchymal stem cell (MSC) markers, including CD105, CD90, CD73, and CD29 (Figures 2(a) and 2(c)). Double immunostaining demonstrated the colocalization of hepatic and MSC markers within the same cells (Figure 2(d)). Hematopoietic

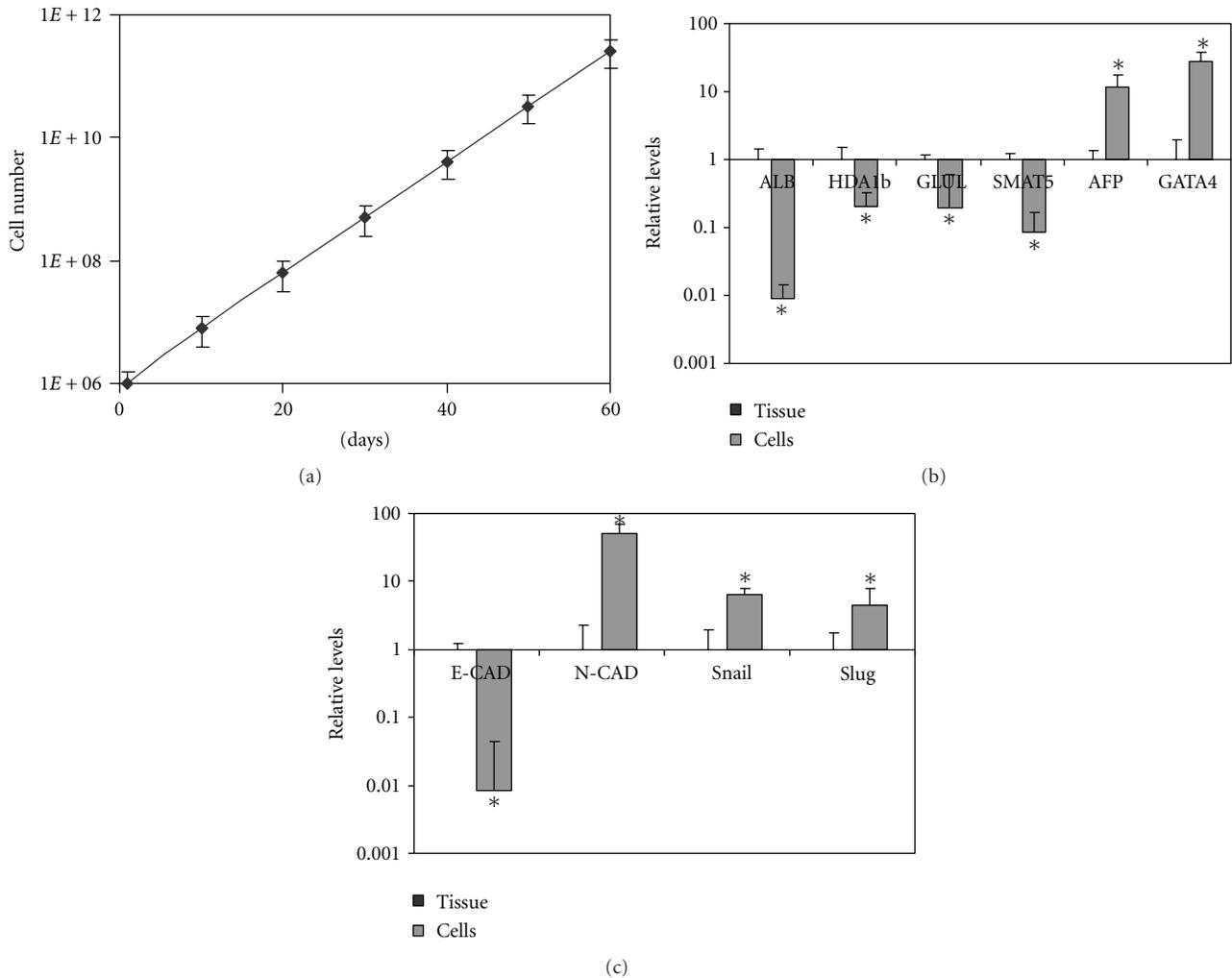


FIGURE 1: Proliferating adult human liver cells in culture undergo hepatic dedifferentiation associated with EMT marker expression. (a) Growth curve of adult human liver cells supplemented with exendin-4 (5 nM) [11]. (b) Quantitative RT-PCR analysis of hepatic (albumin, ADH1b, GLUL, SMAT5, and AFP), endodermal (GATA4) and (c) EMT (E-CAD, N-CAD, Snail, and Slug) markers in primary cultures of adult human liver cells ($n = 4$, P2-4) compared to intact adult human liver tissue ($n = 4$). The results are normalized to β -actin expression within the same cDNA sample and presented as the relative levels of the mean \pm standard deviation of primary cultures versus liver tissue. * $P < 0.01$.

marker expression was not detected in the human liver-derived cultures (Figure 2(a)). These data suggest that liver-derived cells in culture coexpress hepatic and general MSC markers but may not represent a known hepatic progenitor population, as neither CK-19 nor EpCAM expression is detected [28, 36, 37].

3.2. Developmental Plasticity of Liver-Derived Cells in Culture.

The MSC markers present on most liver-derived cells in culture motivated us to analyze whether the cells also exhibit cellular plasticity. Human liver cells at passage 4 were cultured for 21 days under defined differentiation conditions known to activate osteocytes and adipocytes among BM-derived MSCs (Figures 2(e), 2(f), 2(g), and 2(h)). Indeed, human liver cells cultured in adipogenic differentiation medium exhibited lipid-containing droplets visualized by Oil Red staining (Figure 2(e)). Cells cultured in

osteogenic differentiation media developed calcium deposits visualized by Alizarin Red staining, which is characteristic of osteogenic differentiation (Figure 2(g)). These data indicate that the liver-derived cells that propagate *in vitro* acquire developmental plasticity, which may allow them to differentiate along alternate developmental fates in response to applied growth and differentiation conditions. The developmental plasticity these cells exhibited may provide a partial explanation of their capacity to acquire a β -cell phenotype in response to ectopic pancreatic transcription factor expression.

3.3. Liver-Derived Cells Cultured In Vitro Do Not Induce Tumors in Immune Deficient Mice In Vivo .

The epithelial-mesenchymal transition in liver has been suggested to be related to invasiveness and metastatic potential in mouse and human cancers [39, 40]. Moreover, BM-derived

		Expression (Percentile)
Hepatic	Albumin	88 ± 6
	AAT	59 ± 8
	AFP	19 ± 7
	E-Cadherin	7 ± 2
Hepatic progenitors	EpCAM	ND
	NCAM	11 ± 6
	CK19	ND
Mesenchymal stem cells	CA19-9	ND
	CD105	96 ± 2
	CD90	94 ± 4
	CD73	95 ± 3
Hematopoietic	CD29	92 ± 4
	CD45	ND
	CD31	ND
	CD31/CD105	ND
	CD14	ND
	CD11c	ND
	CD34	ND
	MHC Class II	ND
MHC Class I	89 ± 6	

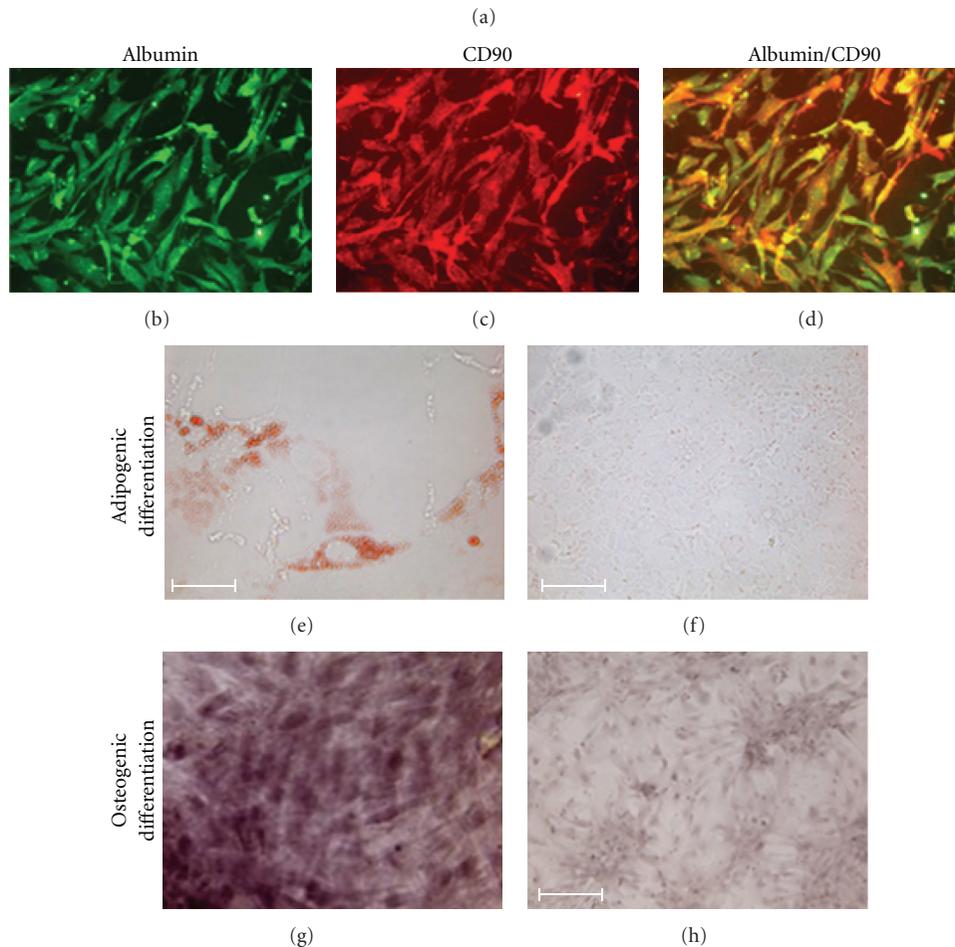


FIGURE 2: Primary adult human liver cell cultures express hepatic and mesenchymal stem-cell markers and differentiate along adipogenic and osteogenic lineages. (a) Adult human liver cells (P1–P7, $n \geq 5$) were characterized for the expression of specific surface and intracellular markers of hepatic, hepatic progenitor, mesenchymal, and hematopoietic lineages by flow cytometry. (b–d) Double immunofluorescent staining of human liver cells (P7) for albumin and CD90. Nuclei were stained by DAPI (blue). Original magnification $\times 20$. (e) Liver cells at P4 were induced to differentiate toward the adipogenic and (g) osteogenic lineages using the specific differentiation cocktails for 21 days in culture. Control cells in regular cell culture media are shown in (f) and (h). Samples were fixed and stained with Oil Red (adipogenic lipids, (e), (f) or Alizarin (osteogenic calcium, (g), (h)). Original magnification $\times 10$.

mesenchymal stem cells have been demonstrated to exhibit tumorigenic capacity upon *in vivo* implantation [41–43]. Therefore, we analyzed whether the implantation of adult human liver-derived cells potentially generates tumors *in vivo* upon transplantation in immune-deficient mice. Adult human liver-derived cells at passage 4 were injected subcutaneously into both flanks of nude mice (1×10^6 cells per injection, 5 mice per group, 2 transplantations per mouse) and tumor growth monitored weekly. Transplantation of tumor-derived cells, such as MDA-MB 231 or Panc-1 cells, resulted in large tumor formation 3–6 weeks after implantation [34], but none of the mice implanted with liver-derived cells developed visible tumors over 4 months. After 17 weeks, the mice were sacrificed and the area of injection examined. No traces of the cells were identified in the specimens. These data suggest that despite the cellular plasticity manifested earlier, dedifferentiated human liver cells have mesenchymal characteristics but may not carry a risk of uncontrolled cell proliferation or tumor formation.

3.4. Irreversible Tracing of Albumin Expression. The adult human-derived liver cells characterized above were reported in the past to undergo cellular reprogramming and generate insulin-producing cells upon ectopic expression of the pancreatic transcription factor PDX-1 [14, 16, 17, 30]. However, because only a fraction of PDX-1 expressing cells become insulin positive [14, 16], we sought to analyze whether the insulin-producing cells are generated from cells that originally express albumin or a yet unidentified side population of stem-like cells that may be enriched in the hepatic-derived primary cultures. Because PDX-1 turns off the hepatic repertoire of gene expression [30], we irreversibly tagged albumin expression prior to PDX-1 treatment. Human liver cells at passages 1–3 were coinfecting by a dual lentivirus system modified from Russ et al. [33]. This lentivirus system included the CMV-loxP-DsRed2-loxP-eGFP (R/G) reporter [33] and an additional lentiviral vector carrying the expression of Cre recombinase under the control of the albumin promoter (ALB-Cre, Figure 3(a)). R/G treatment resulted in DsRed2, but not eGFP, expression in $84.1 \pm 3.1\%$ of cells. The albumin promoter activates the expression of Cre recombinase only in albumin-positive cells. Cre recombinase cleaves the “floxated” DsRed2, allowing the constitutive expression of eGFP under the same CMV promoter (Figure 3). The dual lentivirus system exhibited a high level of specificity; no eGFP-positive cells were detected in non-liver cells, such as pancreatic β TC1 cells (Figures 3(e), 3(f), and 3(g)), and Cre recombinase expression colocalized with albumin (Figures 3(h), 3(i), 3(j), and 3(k)). Because most adult human liver cells express albumin ($88 \pm 6\%$, Figure 2(a)), we expected that the majority of cells infected by both lentiviruses would have activated Cre recombinase and become eGFP-positive. The efficiency of infection with a single lentivirus was $\sim 85\%$, and double infection resulted in 72% eGFP-positive cells, using CMV-Cre as control. Adult human liver cells infected with the two-lentivirus system (R/G and ALB-Cre) resulted in $69.4 \pm 7.6\%$ eGFP-positive cells within ten days of infection, with few cells expressing both eGFP and DsRed2 protein (Figure 3(d)). Colabeling

with eGFP and DsRed2 likely reflects the activity of the albumin promoter and the relatively long half-life of the DsRed2 protein ($t_{1/2} = 4.5$ days), such that the DsRed2 protein can be detected even 1–2 weeks after the DsRed2 gene is no longer expressed [33]. Only $9.3 \pm 5.4\%$ of the cells remained irreversibly positive for DsRed2. These cells, in part, represent an incapability to activate albumin expression (about 2%–3%) and/or cells infected only with the reporter vector CMV-loxP-DsRed2-loxP-eGFP but not by the ALB-Cre lentivirus vector.

3.5. Characterization of Cells Irreversibly Tagged for Albumin Expression by eGFP. eGFP-labeled liver cells were separated from DsRed2-positive cells by FACS-Sorter and cultured separately for several passages (Figures 4(a), 4(b), 4(c), 4(e), and 4(f)). Two weeks after sorting (3 passages), less than 3% of DsRed2-positive cells were detected among the eGFP-positive population, the vast majority of which were colabeled by eGFP (data not shown), suggesting a highly purified culture of albumin-positive, eGFP-labeled cells.

The eGFP and DsRed2 populations exhibited distinct hepatic marker expression. In addition to increased expression of the albumin gene in eGFP-positive cells (Figure 4(g)), additional adult hepatic markers, such as ADH1b, GLUL, and the transcription factor CEBP β , were expressed to higher levels compared to the DsRed2-positive cells (Figure 4(g)). However, the expression level of the hepatic genes in eGFP-positive cells was lower than that of cells at passage 2–4 (Figure 1(b)), further suggesting an ongoing dedifferentiation process which occurs in culture with time. The expression of immature or progenitor markers, such as AFP and CK19, was similarly detected in both groups (data not shown). In contrary, the DsRed2-positive cells were enriched for α SMA, desmin, and GFAP expression compared to the eGFP-positive cells (Figure 4(h)). α SMA, desmin, and GFAP are typically considered to be hepatic stellate cell markers [44]. Although stellate cells are considered mesenchymal cells [45], they do not usually express the mesenchymal marker CD90. The fact that DsRed2 cells also expressed α SMA, desmin, and GFAP but low levels of CD90 (Figure 4(i)) suggests that indeed, the DsRed2 population of cells may include the hepatic stellate cell population. Flow cytometry and immunofluorescence confirmed that each of the isolated eGFP-positive cells expressed albumin, CD105, and CD90 at higher levels than DsRed2-positive cells (Figures 4(j) and 4(k) and data not shown).

3.6. Ectopic PDX-1 Expression Induces Cellular Reprogramming. Next, we sought to analyze which of the two liver cell populations preferentially support PDX-1-induced reprogramming, manifested as induced insulin production. eGFP-positive cells and DsRed2-positive cells were separately treated with *Ad-PDX-1* and supplemented with soluble factors as previously described [16]. Ectopic PDX-1 expression resulted in a significant decrease in albumin gene expression (data not shown) [30]. However, ectopic PDX-1 expression did not affect the expression of CD105 and CD90 in the separate cultures (data not shown). Ectopic PDX-1 expression in eGFP positive cells activated the expression

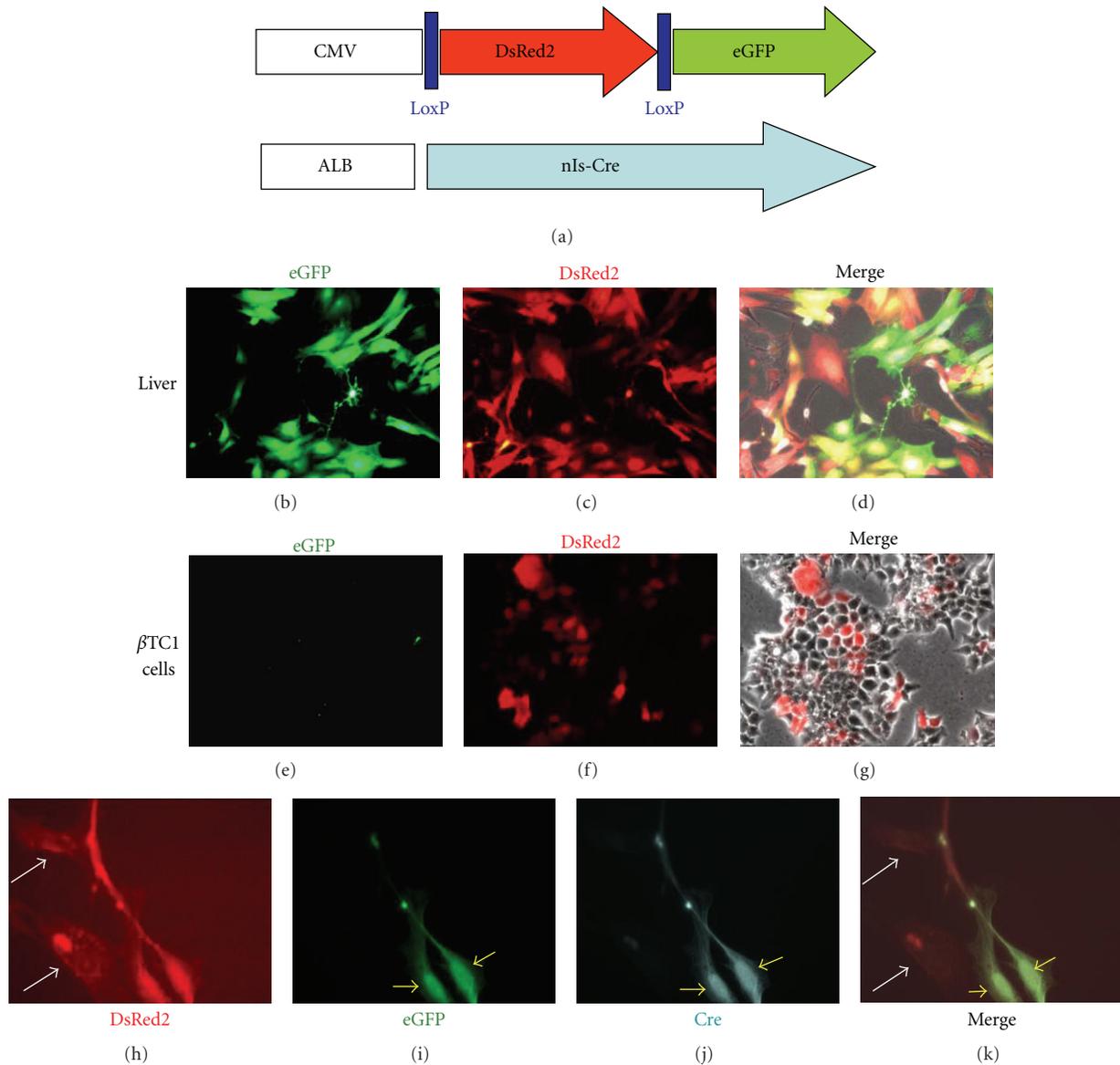


FIGURE 3: Irreversible labeling for albumin expression using the dual lentivirus system (lineage tracing). (a) Schematic presentation of the lentivirus vectors. (b–d) Adult human liver cells or (e–g) β -TC1 cells were infected with the reporter virus in combination with ALB-Cre virus. Liver cells were imaged 10 days after infection for DsRed2 (red) or eGFP (green) autofluorescence (original magnification $\times 10$). (h–k) Immunostaining of adult human cells infected with both viruses for Cre (blue, (j)), eGFP (green, (i)), and DsRed2 (red, (h)). (k) Merged image of all stainings. White arrows indicate DsRed2-positive cells. Yellow arrows indicate eGFP and Cre double positive cells. Original magnification $\times 60$.

of pancreatic hormones gene expression (INS, GCG, and SST; Figure 5(a)), the expression of genes involved in β -cell glucose sensing (GLUT-2, GK), and prohormone processing (PC2) (Figure 5(b)). Only the expression of somatostatin was significantly activated in DsRed2 positive cells. Moreover, PDX-1 treatment resulted in insulin production, which mainly colocalized with eGFP; $17.6 \pm 3.5\%$ of eGFP-positive cells coexpressed insulin (Figures 5(c)–5(h)). In contrast, only $3.35 \pm 2.1\%$ of DsRed2-positive cells was also positive for insulin in response to a similar reprogramming protocol (data not shown). Because about 28% of the DsRed2-positive cells could have been positive for albumin but not eGFP,

the actual percentage of insulin-positive cells generated in albumin-negative human liver cells is even lower than $3.35 \pm 2.1\%$. Taken together, these data suggest that albumin-positive cells represent the vast majority of liver cells that undergo reprogramming along the pancreatic lineage.

4. Discussion

Using a genetic lineage-tracing approach, we demonstrate for the first time that the insulin-producing cells induced by ectopic expression of the pancreatic transcription factor PDX-1 in human liver cells mainly originate from

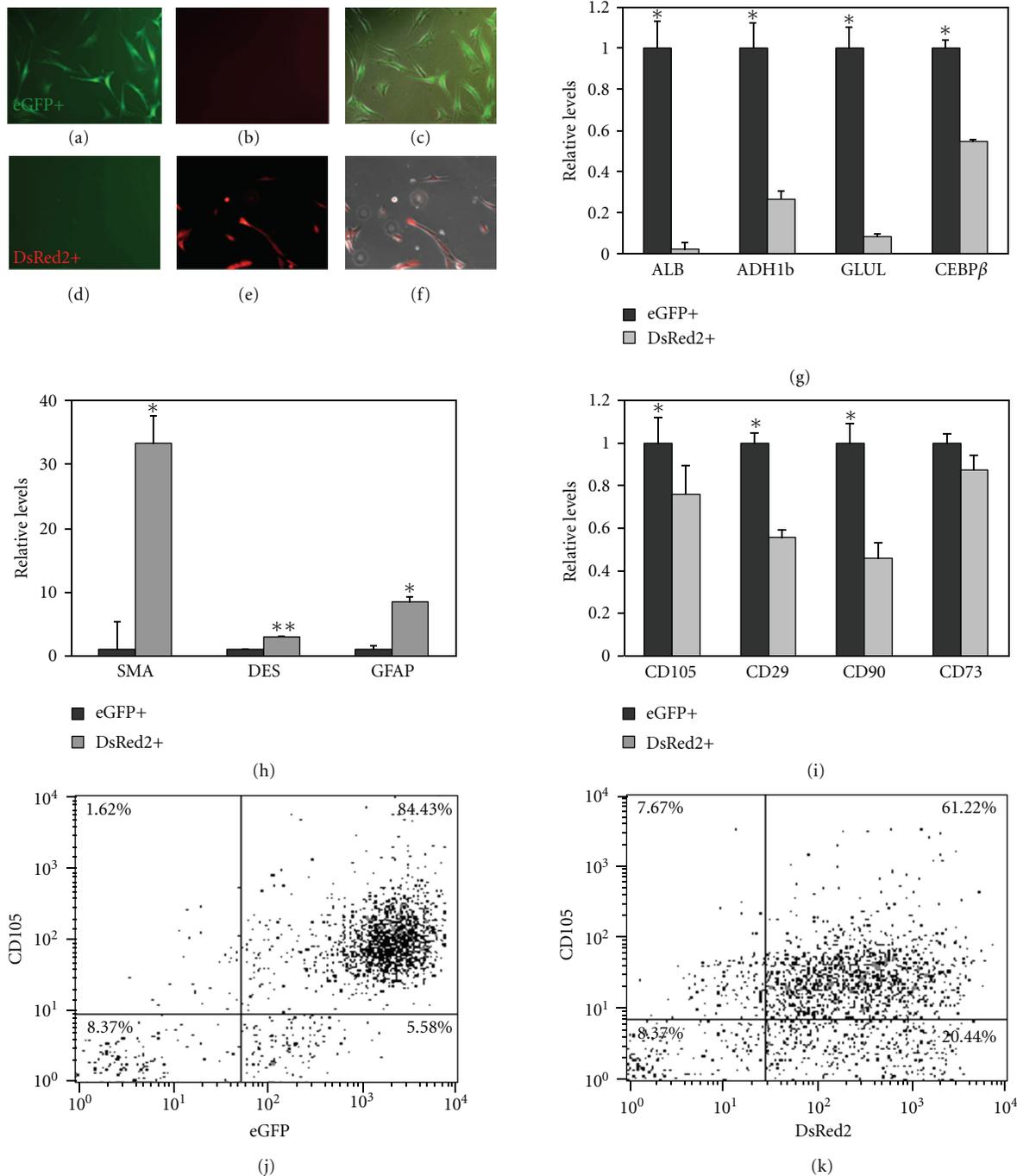


FIGURE 4: Isolated cultures of eGFP-positive cells express hepatic markers. Adult human liver cells were infected with the reporter virus and ALB-Cre virus. Two weeks later, eGFP-positive and DsRed2-positive cells were separated using a cell sorter. The eGFP-positive (a–c) and DsRed2-positive (d–f) cells were separately cultured and imaged 24 hours later for eGFP ((a) and (d), green) and DsRed2 ((b) and (e), red) fluorescence. (c and f) eGFP and DsRed2 merge images. Original magnification $\times 20$. Quantitative RT-PCR in eGFP-positive cells compared to DsRed2-positive cells using mRNA extracted 2 weeks after sorting of (g) hepatic (albumin, ADH1b, GLUL, and CEBP β), (h) stellate cell (SMA, desmin, and GFAP), and (i) mesenchymal stem cell (CD105, CD29, CD90, and CD73) marker expression. The results were normalized to β -actin expression within the same cDNA sample and presented as the relative levels of the mean \pm standard deviation of DsRed2-positive versus eGFP-positive cells, $n = 6$ from 3 different cultures. * $P < 0.01$, ** $P < 0.05$. (j) The expression of the mesenchymal marker CD105 was analyzed in the eGFP and (k) DsRed2-positive cells by flow cytometry.

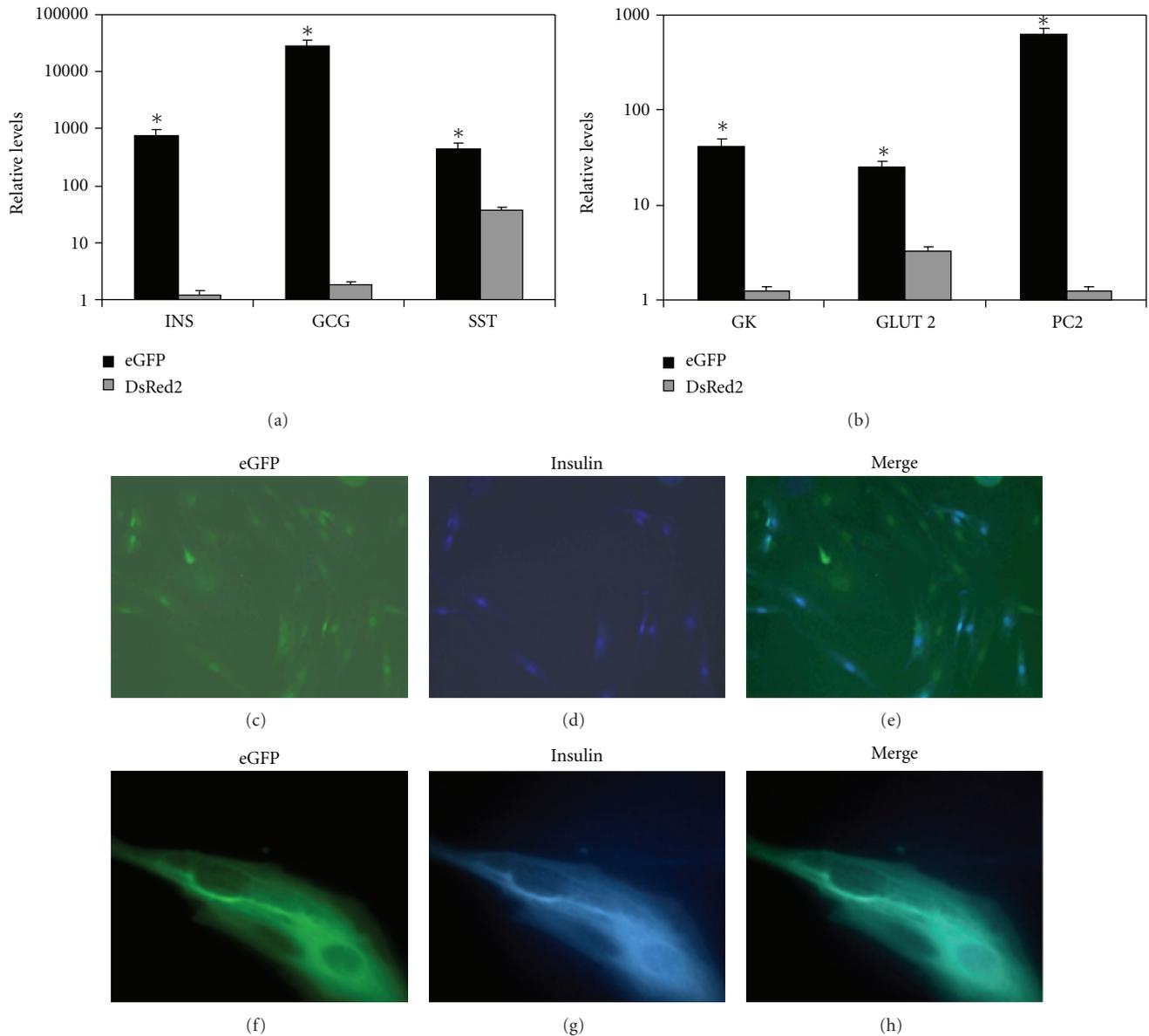


FIGURE 5: Ectopic PDX-1 expression induced pancreatic differentiation mainly in eGFP positive liver cells. eGFP-positive cells and DsRed2-positive cells were treated with *Ad*-PDX-1 and soluble factors for 5 days. Quantitative RT-PCR analysis of (a) pancreatic hormones (INS, GG, and SST), and (b) β -cell specific genes (GK, GLUT2, and PC2) in eGFP-positive cells and DsRed2-positive cells ($n = 2$ p7–9). The results are normalized to β -actin expression within the same cDNA sample and presented as the relative levels of the mean \pm standard deviation of PDX-1 treated cells versus control *Ad*- β -gal treated cells. * $P < 0.01$. (c–h) Double immunofluorescence analysis of GFP with insulin in *Ad*-PDX-1 treated eGFP-positive liver cells. Original magnification $\times 20$ (c–e) and $\times 60$ (f–h). Five hundred cells were analyzed under the fluorescent microscope for eGFP, DsRed2, or insulin in three different cultures.

albumin-positive cells. The proliferating adherent human liver cells express both adult liver and mesenchymal cell markers and possess a considerable level of developmental plasticity (Figures 1 and 2).

Most liver-derived cells in the primary culture express mesenchymal characteristics (Figure 2). The origin of these hepatic MSC-like cells is not clear; they could represent liver cells that underwent EMT, or the cells may represent a preexisting population of stem-like cells with self-replication capacity, which normally serve as hepatic progenitor cells.

The precise combination of markers that characterize human hepatic progenitor cells is controversial [29]; thus, it is complicated to completely rule out the possibility that our culture conditions promoted the amplification of pre-existing human hepatic stem-like cells. However, the comprehensive characterization of the primary culture of the human liver-derived cells seems to favor the option that insulin-producing cells are generated in dedifferentiated liver cells that underwent an EMT process, meaning that such cells, most likely, do not exist in the intact organ. This

conclusion is based on several lines of evidence. First, 88% of the cells in culture are not only positive for albumin, but the albumin-positive cells also express higher levels of other adult hepatic markers, which is not expected to occur in pluripotent stem-like cells. Second, EpCAM is agreed upon as being a hepatic progenitor marker and is not expressed in our human-derived cells. Third, the only cells in the intact adult liver with mesenchymal characteristics are stellate cells. However, stellate cell marker expression was low in general and occurred mainly in the albumin-negative population, which had lower reprogramming efficiency (Figure 4(h)). Taken together, these data may suggest that the cells populating the majority of human liver-derived primary culture are not residents of the intact organ that underwent preferential proliferation. On the other hand, our human liver-derived primary culture exhibits several EMT characteristics, including specific mesenchymal marker expression (Figures 1 and 2), decreased E-CAD associated with increased N-CAD expression (Figure 1(b)), and the activation of Snail and Slug expression, the zinc finger transcription factors known to control the EMT process (Figure 1(c)) [40, 46].

Epithelial to mesenchymal transition is a common process that epithelial cells undergo upon *in vitro* culture [47–49]. *In vivo*, EMT plays a key role in morphogenic changes during embryonic development, wound healing/tissue regeneration, and neoplasia [25, 26, 30]. Several groups have demonstrated that the process is associated with the downregulation of epithelial gene expression and activation of mesenchymal gene expression [47–49]. EMT induction *in vitro* has been suggested in cultured thyroid cells [50] and adult human islets upon entrance into the cell cycle [51]. More recently, direct evidence of EMT in cultured adult primary β -cells was demonstrated using genetic lineage tracing [38]. The capacity of adult parenchymal liver cells to give rise to insulin-producing cells is further strengthened by *in vivo* studies. Direct administration of PDX-1 in mice *in vivo* suggests that the insulin-producing cells are primarily generated in parenchymal cells close to the central veins [8, 11, 25]. Hepatic pericentral cells are suggested adult, terminally differentiated, while adult hepatic stem cells mainly reside in periportal areas of the liver and in the Canals of Hering [22]. While these observations need to be directly analyzed by a lineage tracing approach, they suggest that the reprogramming process does not primarily take place in *bona fide* adult hepatic stem cells.

Despite the uniform morphology of the human liver-derived cells *in vitro*, the cells seem to maintain different phenotypes with regard to albumin and the expression of other adult hepatic markers, which may correlate with distinct reprogramming capacity. Using irreversible lineage tracing for albumin promoter activity, we present supporting evidence that insulin-positive cells induced by ectopic PDX-1 expression are preferentially generated in cells that are originally albumin-positive (Figure 5). In a recent paper, Dan et al. [28] isolated human hepatic progenitor cells that carry the capacity to differentiate along multiple hepatic lineages, including mesenchymal cells. However, these cells did not express albumin. The colocalization of albumin expression

with mesenchymal markers suggests that the reprogramming process of liver to pancreas does not occur in such cells but in hepatic dedifferentiated cells [30], which undergone an EMT process.

Because we also detected a small number of insulin-positive cells among the DsRed2-positive cells, we cannot rule out the possibility that nonalbumin-positive cells present in our culture underwent reprogramming and contributed to the insulin-producing cell population but with lower reprogramming efficiency.

The fact that the special mesenchymal cells coexpressing albumin do not induce tumors in immune-deficient rodents conveys a substantial safety advantage over using other progenitor cells in tissue engineering approaches [52, 53]. By contrast, other mesenchymal cells derived from bone marrow have been documented in numerous studies to carry a tumorigenic potential under similar conditions [41–43].

The data generated in this study provide a better understanding of the nature of primary human liver cell culture, which is capable of undergoing pancreatic transcription factor-induced reprogramming. Further dissection of subpopulations of cells within the liver-derived, albumin-positive mesenchymal cells will allow us to identify the specific characteristics of liver cells, which are predisposed to reprogramming to allow a substantial increase in the efficiency of the reprogramming process along the pancreatic lineage.

5. Conclusion and Summary

The present study suggests that the insulin-producing cells induced by ectopic expression of the pancreatic transcription factor PDX-1 in human liver cells mainly originate from albumin-positive cells. The proliferating adult human liver cells in culture acquire mesenchymal characteristics and a high level of cellular plasticity. However, while most liver cells in culture possess mesenchymal-like characteristics, insulin production is induced in albumin positive cells, which are enriched for adult hepatic markers expression. Identification and the characterization of cells prone to reprogramming along the β -cell lineage is expected to increase the reprogramming efficiency. It may allow developing controlled and reproducible reprogramming process, by overcoming the pronounced heterogeneity of cells in the primary cultures.

Reprogramming liver to pancreas offers the access to an abundant source of “self-tissue”. The approach obviates the shortage in tissue availability from cadaveric donors and the need for antirejection treatment, allowing the diabetic patient to be the donor of his own therapeutic tissue.

Acknowledgments

The authors thank Tamar Rubinek, Lilach Abramovitz, and Hagai Ligumsky for their guidance and assistance in performing the *in vivo* studies. They thank Dora Berman-Weinberg for her support of the *in vitro* studies and Kfir Molakandov, Keren Shternhall-Ron, and Vered Aviv for fruitful discussions, and Michael Walker and Shimon Efrat for critically reviewing the paper. This study was supported

by Juvenile Diabetes Research Foundation no. 17-2006-1029. The authors declare no conflict of interest in their paper. This work was performed in partial fulfillment of the requirements for Ph.D. degree.

References

- [1] S. Bonner-Weir and G. C. Weir, "New sources of pancreatic β -cells," *Nature Biotechnology*, vol. 23, no. 7, pp. 857–861, 2005.
- [2] I. Meivar-Levy and S. Ferber, "Regenerative medicine: using liver to generate pancreas for treating diabetes," *Israel Medical Association Journal*, vol. 8, no. 6, pp. 430–434, 2006.
- [3] C. A. Palma, R. Lindeman, and B. E. Tuch, "Blood into β -cells: an adult stem cells be used as a therapy for Type 1 diabetes?" *Regenerative Medicine*, vol. 3, no. 1, pp. 33–47, 2008.
- [4] K. S. Zaret and M. Grompe, "Generation and regeneration of cells of the liver and pancreas," *Science*, vol. 322, no. 5907, pp. 1490–1494, 2008.
- [5] G. Deutsch, J. Jung, M. Zheng, J. L6ra, and K. S. Zaret, "A bipotential precursor population for pancreas and liver within the embryonic endoderm," *Development*, vol. 128, no. 6, pp. 871–881, 2001.
- [6] J. K. Reddy, S. Rao, A. V. Yeldandi, X. Tan, and R. S. Dwivedi, "Pancreatic hepatocytes. An in vivo model for cell lineage in pancreas of adult rat," *Digestive Diseases and Sciences*, vol. 36, no. 4, pp. 502–509, 1991.
- [7] M. D. Dabeva, S. G. Hwang, S. R. G. Vasa et al., "Differentiation of pancreatic epithelial progenitor cells into hepatocytes following transplantation into rat liver," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 14, pp. 7356–7361, 1997.
- [8] I. Ber, K. Shternhall, S. Perl et al., "Functional, persistent, and extended liver to pancreas transdifferentiation," *Journal of Biological Chemistry*, vol. 278, no. 34, pp. 31950–31957, 2003.
- [9] L. Z. Cao, D. Q. Tang, M. E. Horb, S. W. Li, and L. J. Yang, "High glucose is necessary for complete maturation of Pdx1-VP16-expressing hepatic cells into functional insulin-producing cells," *Diabetes*, vol. 53, no. 12, pp. 3168–3178, 2004.
- [10] S. Ferber, A. Halkin, H. Cohen et al., "Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia," *Nature Medicine*, vol. 6, no. 5, pp. 568–572, 2000.
- [11] M. Koizumi, R. Doi, E. Toyoda et al., "Hepatic regeneration and enforced PDX-1 expression accelerate transdifferentiation in liver," *Surgery*, vol. 136, no. 2, pp. 449–457, 2004.
- [12] K. Shternhall-Ron, F. J. Quintana, S. Perl et al., "Ectopic PDX-1 expression in liver ameliorates type 1 diabetes," *Journal of Autoimmunity*, vol. 28, no. 2-3, pp. 134–142, 2007.
- [13] D. Q. Tang, S. Lu, Y. P. Sun et al., "Reprogramming liver-stem WB cells into functional insulin-producing cells by persistent expression of Pdx1- and Pdx1-VP16 mediated by lentiviral vectors," *Laboratory Investigation*, vol. 86, no. 1, pp. 83–93, 2006.
- [14] V. Aviv, I. Meivar-Levy, I. H. Rachmut, T. Rubinek, E. Mor, and S. Ferber, "Exendin-4 promotes liver cell proliferation and enhances PDX-1-induced liver to pancreas transdifferentiation," *Journal of Biological Chemistry*, vol. 284, no. 48, pp. 33509–33520, 2009.
- [15] S. Gefen-Halevi, I. H. Rachmut, K. Molakandov et al., "NKX6.1 promotes PDX-1-induced liver to pancreatic β -cells reprogramming," *Cellular Reprogramming*, vol. 12, no. 6, pp. 655–664, 2010.
- [16] T. Sapir, K. Shternhall, I. Meivar-Levy et al., "From the cover: cell-replacement therapy for diabetes: generating functional insulin-producing tissue from adult human liver cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 22, pp. 7964–7969, 2005.
- [17] M. Zalzman, S. Gupta, R. K. Giri et al., "Reversal of hyperglycemia in mice by using human expandable insulin-producing cells differentiated from fetal liver progenitor cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 12, pp. 7253–7258, 2003.
- [18] M. Zalzman, L. Anker-Kitai, and S. Efrat, "Differentiation of human liver-derived, insulin-producing cells toward the β -cell phenotype," *Diabetes*, vol. 54, no. 9, pp. 2568–2575, 2005.
- [19] W. C. Li, M. E. Horb, D. Tosh, and J. M. W. Slack, "In vitro transdifferentiation of hepatoma cells into functional pancreatic cells," *Mechanisms of Development*, vol. 122, no. 6, pp. 835–847, 2005.
- [20] I. M. Arias, J. L. Boyer, F. V. Chisari, N. Fausto, D. Schachter, and D. A. Shafritz, *The Liver: Biology and Pathobiology*, Williams & Wilkins, Philadelphia, Pa, USA, 4th edition, 2001.
- [21] E. Gaudio, G. Carpino, V. Cardinale, A. Franchitto, P. Onori, and D. Alvaro, "New insights into liver stem cells," *Digestive and Liver Disease*, vol. 41, no. 7, pp. 455–462, 2009.
- [22] R. Turner, O. Lozoya, Y. Wang et al., "Human hepatic stem cell and maturational liver lineage biology," *Hepatology*, vol. 53, no. 3, pp. 1035–1045, 2011.
- [23] H. Kojima, M. Fujimiya, K. Matsumura, T. Nakahara, M. Hara, and L. Chan, "Extrapancreatic insulin-producing cells in multiple organs in diabetes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 8, pp. 2458–2463, 2004.
- [24] H. Kojima, M. Fujimiya, K. Matsumura et al., "NeuroD-beta-cellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice," *Nature Medicine*, vol. 9, no. 5, pp. 596–603, 2003.
- [25] J. Imai, H. Katagiri, T. Yamada et al., "Constitutively active PDX1 induced efficient insulin production in adult murine liver," *Biochemical and Biophysical Research Communications*, vol. 326, no. 2, pp. 402–409, 2005.
- [26] V. Yechoor, V. Liu, C. Espiritu et al., "Neurogenin3 is sufficient for transdetermination of hepatic progenitor cells into neo-islets in vivo but not transdifferentiation of hepatocytes," *Developmental Cell*, vol. 16, no. 3, pp. 358–373, 2009.
- [27] E. Schmelzer, E. Wauthier, and L. M. Reid, "The phenotypes of pluripotent human hepatic progenitors," *Stem Cells*, vol. 24, no. 8, pp. 1852–1858, 2006.
- [28] Y. Y. Dan, K. J. Riehle, C. Lazaro et al., "Isolation of multipotent progenitor cells from human fetal liver capable of differentiating into liver and mesenchymal lineages," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 26, pp. 9912–9917, 2006.
- [29] J. Jozefczuk, H. Stachelscheid, L. Chavez et al., "Molecular characterization of cultured adult human liver progenitor cells," *Tissue Engineering—Part C*, vol. 16, no. 5, pp. 821–834, 2010.
- [30] I. Meivar-Levy, T. Sapir, S. Gefen-Halevi et al., "Pancreatic and duodenal homeobox gene 1 induces hepatic dedifferentiation by suppressing the expression of CCAAT/enhancer-binding protein β ," *Hepatology*, vol. 46, no. 3, pp. 898–905, 2007.
- [31] H. M. Kim, S. B. Han, B. H. Hyun et al., "Functional human hepatocytes: isolation from small liver biopsy samples and primary cultivation with liver-specific functions," *Journal of Toxicological Sciences*, vol. 20, no. 5, pp. 565–578, 1995.

- [32] E. Thévenot, F. Côté, P. Colin et al., "Targeting conditional gene modification into the serotonin neurons of the dorsal raphe nucleus by viral delivery of the Cre recombinase," *Molecular and Cellular Neuroscience*, vol. 24, no. 1, pp. 139–147, 2003.
- [33] H. A. Russ, Y. Bar, P. Ravassard, and S. Efrat, "In vitro proliferation of cells derived from adult human β -cells revealed by cell-lineage tracing," *Diabetes*, vol. 57, no. 6, pp. 1575–1583, 2008.
- [34] I. Wolf, J. O'Kelly, N. Wakimoto et al., "Honokiol, a natural biphenyl, inhibits in vitro and in vivo growth of breast cancer through induction of apoptosis and cell cycle arrest," *International Journal of Oncology*, vol. 30, no. 6, pp. 1529–1537, 2007.
- [35] Y. Sakai, S. Yamagami, and K. Nakazawa, "Comparative analysis of gene expression in rat liver tissue and monolayer- and spheroid-cultured hepatocytes," *Cells Tissues Organs*, vol. 191, no. 4, pp. 281–288, 2010.
- [36] M. Inada, D. Benten, K. Cheng et al., "Stage-specific regulation of adhesion molecule expression segregates epithelial stem/progenitor cells in fetal and adult human livers," *Hepatology International*, vol. 2, no. 1, pp. 50–62, 2008.
- [37] M. E. Fomin, L. K. Tai, A. Barcena, and M. O. Muench, "Coexpression of CD14 and CD326 discriminate hepatic precursors in the human fetal liver," *Stem Cells and Development*, vol. 2010, p. 2, 2010.
- [38] H. A. Russ, P. Ravassard, J. Kerr-Conte, F. Pattou, and S. Efrat, "Epithelial-mesenchymal transition in cells expanded in vitro from lineage-traced adult human pancreatic beta cells," *PLoS One*, vol. 4, no. 7, Article ID e6417, 2009.
- [39] W. Ding, H. You, H. Dang et al., "Epithelial-to-mesenchymal transition of murine liver tumor cells promotes invasion," *Hepatology*, vol. 52, no. 3, pp. 945–953, 2010.
- [40] M. Zeisberg and E. G. Neilson, "Biomarkers for epithelial-mesenchymal transitions," *Journal of Clinical Investigation*, vol. 119, no. 6, pp. 1429–1437, 2009.
- [41] M. Miura, Y. Miura, H. M. Padilla-Nash et al., "Accumulated chromosomal instability in murine bone marrow mesenchymal stem cells leads to malignant transformation," *Stem Cells*, vol. 24, no. 4, pp. 1095–1103, 2006.
- [42] N. Serakinci, P. Guldborg, J. S. Burns et al., "Adult human mesenchymal stem cell as a target for neoplastic transformation," *Oncogene*, vol. 23, no. 29, pp. 5095–5098, 2004.
- [43] J. Tolar, A. J. Nauta, M. J. Osborn et al., "Sarcoma derived from cultured mesenchymal stem cells," *Stem Cells*, vol. 25, no. 2, pp. 371–379, 2007.
- [44] S. L. Friedman, "Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver," *Physiological Reviews*, vol. 88, no. 1, pp. 125–172, 2008.
- [45] J. Dudas, T. Mansuroglu, D. Batusic, B. Saile, and G. Ramadori, "Thy-1 is an in vivo and in vitro marker of liver myofibroblasts," *Cell and Tissue Research*, vol. 329, no. 3, pp. 503–514, 2007.
- [46] R. Kalluri and R. A. Weinberg, "The basics of epithelial-mesenchymal transition," *Journal of Clinical Investigation*, vol. 119, no. 6, pp. 1420–1428, 2009.
- [47] S. S. Choi and A. M. Diehl, "Epithelial-to-mesenchymal transitions in the liver," *Hepatology*, vol. 50, no. 6, pp. 2007–2013, 2009.
- [48] A. Kaimori, J. Potter, J. Y. Kaimori, C. Wang, E. Mezey, and A. Koteish, "Transforming growth factor- β 1 induces an epithelial-to-mesenchymal transition state in mouse hepatocytes in vitro," *Journal of Biological Chemistry*, vol. 282, no. 30, pp. 22089–22101, 2007.
- [49] M. Zeisberg, C. Yang, M. Martino et al., "Fibroblasts derive from hepatocytes in liver fibrosis via epithelial to mesenchymal transition," *Journal of Biological Chemistry*, vol. 282, no. 32, pp. 23337–23347, 2007.
- [50] L. Ulianich, C. Garbi, A. S. Treglia et al., "ER stress is associated with dedifferentiation and an epithelial-to-mesenchymal transition-like phenotype in PC Cl3 thyroid cells," *Journal of Cell Science*, vol. 121, no. 4, pp. 477–486, 2008.
- [51] M. C. Gershengorn, A. A. Hardikar, C. Wei, E. Ceras-Raaka, B. Marcus-Samuels, and B. M. Raaka, "Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells," *Science*, vol. 306, no. 5705, pp. 2261–2264, 2004.
- [52] E. E. Baetge, "Production of beta-cells from human embryonic stem cells," *Diabetes, Obesity and Metabolism*, vol. 10, 4, pp. 186–194, 2008.
- [53] D. Zhang, W. Jiang, M. Liu et al., "Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells," *Cell Research*, vol. 19, no. 4, pp. 429–438, 2009.