Cell-based therapy of diabetes: What are the new sources of beta cells?

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Received 2 February 2011; received in revised form 23 May 2011; accepted 24 May 2011
Available online 21 July 2011

Abstract
Diabetes affects 246 million people around the world. To date, no definitive cure has been discovered. Recent clinical trials have shed light on the possibility of successfully transplanting adult pancreatic islets into type 1 diabetic recipients. However, despite encouraging efforts to improve such protocols, the poor availability of pancreatic islets remains a limiting parameter for these transplantation programmes. In the present review, different strategies to obtain other sources of islet beta cells are discussed.

Keywords: Pancreas; Beta cells; Development; Stem cells; Review

Résumé
Thérapie cellulaire du diabète : quelles sont les nouvelles sources de cellules bêta?

Le diabète atteint 246 millions d’individus dans le monde. Des essais cliniques récents ont mis en évidence la possibilité de transplanter avec succès des îlots chez des patients diabétiques de type 1. Malgré les efforts encourageants pour améliorer de tels protocoles, la faible disponibilité des îlots reste un paramètre limitant pour ces programmes de transplantation. Dans la présente revue, nous discutons les différentes stratégies pour obtenir d’autres sources de cellules bêta.

Mots clés : Pancréas ; Cellules bêta ; Développement ; Cellules souches ; Revue

1. Introduction

Diabetes mellitus leads to hyperglycaemia and abnormal beta-cell function. At least 200 million people worldwide suffer from diabetes. Type 1 diabetes, also known as “insulin-dependent diabetes”, is an autoimmune disease that represents 10% of all diabetic patients. To date, diabetes can be treated, but not permanently cured. However, new strategies are being developed to improve the preservation of beta-cells, allow independence from insulin treatment and avoid the complications of type 1 diabetes, including heart disease, kidney disease and retinopathy.

The possibility of beta-cell replacement by transplantation of mature islets was first raised by Shapiro et al. [1] (Fig. 1A). Islets from cadaveric donors were harvested, purified and then transplanted by transhepatic portal injection. Transplanted patients received a glucocorticoid-free immunosuppressive regimen consisting of sirolimus, tacrolimus and daclizumab. Most of the patients were normoglycaemic and independent of insulin injections after the treatment. However, a 5-year follow-up of that clinical study indicated that the treatment was not stable in the long term [2]. Numerous laboratories around the world are now attempting to improve on the protocol to obtain a longer duration of insulin independency. Recently, Vantyghem et al. [3] investigated the role of primary graft function in the duration of graft activity. In this work, the authors performed two or three sequential islet infusions, and primary graft function was evaluated by measuring the beta score, a validated index based on insulin treatment requirement, plasma C-peptide, blood glu-

**Abbreviations:** pO2, Oxygen tension; HIF, Hypoxia inducible factor; VHL, Von Hippel-Lindau; PDX1, Pancreatic and duodental homebox factor 1.
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doi:10.1016/j.diabet.2011.05.007
A. Soggia et al. / Diabetes & Metabolism 37 (2011) 371–375

Cose and HbA1c. After a 3.3-year follow-up, 57% (n = 8) of the patients remained insulin-independent, with HbA1c < 6 and 78% with optimal primary graft function [3]. Such results support the fact that optimal primary graft function can prolong graft survival and function. Islet transplantation thus appears to be a promising strategy, although one major limitation appears to be the poor availability of islets from donors [4].

The need for islet sources for transplantation has prompted research into other sources of pancreatic islets (Fig. 1A). One possibility is the use of animal islet cells, and the most relevant candidate is pig. However, the problem of xenozoonoses has delayed clinical trials. Recently, Garkavenko et al. [5] investigated the potential of infection by xenotic viruses – namely, porcine endogenous retrovirus (PERV), porcine lymphotropic herpes virus (PLHV), porcine cytomegalovirus (PCMV) and porcine circovirus (PCV) – in pig-to-primate xenotransplantation. Islets were prepared from 5- to 7-day-old pigs derived from a specially designed pathogen-free herd. This preclinical study demonstrated the absence of xenotic virus transmission from pigs to primates. More recently, a clinical trial using pig islets was performed (Elliott et al., 70th Scientific Sessions of the American Diabetes Association, June 25–29, 2010, Orlando, FL; abstract no. 161-OR). Seven type 1 diabetic patients, aged 23–63 years, received a transplantation of 5000–10,000 pig alginate-encapsulated islet equivalents per kg body mass. Six months after the transplant, two patients were euglycaemic and insulin-independent. In the remaining patients, the dose of injected insulin was reduced by 34%. Taken altogether, these trials indicate the possibility of protecting recipients against porcine infection while maintaining pancreatic islet function. However, a long-term follow-up is required to monitor the eventual immune rejection of the grafts.

Several possibilities are being considered to generate beta-cells, including embryonic stem cells, adult stem cells and differentiated cells of other types that could be directed towards an insulin-producing cell fate.

2. Embryonic stem cells

Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of the blastocyst. They are able, in theory, to give rise to all cell types, including pancreatic beta-cells. One advantage of these cells is their unlimited capacity for self-renewal. Recently, it was shown that pancreatic endoderm can be derived in vitro from human ES cells under specific culture conditions. Interestingly, such pancreatic endoderm generates glucose-responsive endocrine cells after implantation into mice [6]. These insulin-producing cells express transcription factors that are specific of mature beta-cells, and release human C-peptide in response to glucose [6]. However, the strategy has still not been completely optimalized. Indeed, the development of teratomas is frequently observed when pancreatic endoderm derived from human ES cells is grafted into recipient mice. This problem prevents the possibility of clinical trials at present, but...
the encouraging results [6–8] are probably worth being improved upon in the future.

Methods of beta-cell generation from stem cells have been widely investigated. The aim of these protocols is to reproduce the different stages of embryonic development. Interestingly, a pharmacological strategy was used to screen compounds that direct the differentiation of human stem cells into definitive endoderm. In a screen of 4000 compounds, two of them, termed “IDE1” and “IDE2”, were selected for their ability to induce endodermal differentiation [9]. Another small molecule, indolactam V, was also found to induce differentiation of endoderm into Pdx1-expressing pancreatic progenitors [10]. Such advances should provide further tools to increase our capacity to derive beta-cells in vitro.

3. Cell reprogramming

A recent strategy aimed to generate pluripotent stem cells from type 1 diabetic patients. To this end, fibroblasts from diabetic patients were reprogrammed in diabetes-induced pluripotent stem cells (DiPS) [11]. Fibroblasts were first cultured and infected with retroviruses encoding the transcription factors OCT4, SOX2 and KLF4 that confer pluripotency to fibroblasts [12]. When cultured under specific conditions, the DiPS differentiated into insulin-producing cells that, in turn, were able to respond to glucose [11]. The culture protocol mimicked the different steps of pancreatic development: the differentiation of DiPS was directed to definitive endoderm, followed by gut endoderm, pancreatic progenitors and, finally, beta-like cells [11]. The advantage of such a strategy is to derive beta cells with maximum immunocompatibility with the patient. However, recent research suggests that reprogrammed cells suffer from “genomic instability” that confers tumorigenic potential [13]. Indeed, typical stem-cell reprogramming protocols involve the expression of a set of genes, including the proto-oncogenes c-myc and KLF4. Furthermore, these protocols induce genomic deletions and amplifications. Moreover, the threat of autoimmunity that could be directed against these generated beta-cells is not excluded and needs to be examined. Thus, induced pluripotent stem cell (iPS) technology requires further detailed investigation before considering its use in regenerative medicine.

4. Generation of insulin-producing cells from other cell types

Over the past decade, the differentiation of beta cells from extrapancreatic organs has been described (Fig. 1B); studies have involved bone marrow, pancreatic mesenchymal stem cells, pancreatic duct cells, umbilical cord and liver cells [14–16]. The human umbilical cord has been described as a reservoir of stem cells that could differentiate into ectodermal, mesodermal and endodermal lineages [17–19]. Recently, Kadam and Bhonde [20] isolated mesenchymal stem cells from human umbilical cord. These cells expressed CD29, CD44, CD73, CD90, CD117, smooth muscle actin, nestin, vimentin, proliferation marker Ki67, and the embryonic markers Oct4 and SSEA4. Under specific culture conditions, these cells differentiated into fat, cartilage, neurons and islet-like clusters (ILCs), the latter expressing human C-peptide, insulin and glucagon. When ILCs were transplanted into diabetic mice, normoglycaemia was restored. Mesenchymal stem cells have also been found within pancreatic islets [21–23]. As mesenchymal cells have a high proliferative capacity, they represent an interesting source of islet cells that should be further examined.

The presence of beta-cell progenitors in pancreatic ducts has been widely studied over the past 20 years [24]. Several recent studies indicate that duct cells could be a source of beta-cells during regeneration of the adult pancreas. After partial pancreatectomy, rat pancreatic duct cells dedifferentiated, as demonstrated by the loss of expression of the HNF6 marker. These duct cells constitute regenerating foci that include proliferating ductules, which are positive for Pdx1, Sox9 and Tcf2, markers of the undifferentiated embryonic pancreas [25]. In addition, these duct cells transiently express Ngn3 in the regenerating foci and form new islets. In these experiments, ductal cells recapitulated embryonic beta-cell differentiation. In another experimental model, it was shown that duct ligation of the adult mouse pancreas leads to beta-cell neogenesis [26]. In this duct-ligation model, expression of Ngn3 was also observed in pancreatic ducts. Taken altogether, these data support the fact that plasticity of the pancreas is preserved during adulthood, and that regenerative processes can be stimulated by specific injuries.

In 2000, Ferber et al. [27] showed that ectopic expression of the transcription factor termed “pancreatic and duodenal homeobox factor 1” (Pdx1) was able to induce expression of insulin in the oval cells of the liver. More recently, it was shown that Nkx6.1, another transcription factor uniquely expressed by beta cells in the adult pancreas, was able to induce the expression of early factors of pancreatic differentiation – namely, Ngn3 and Is1 – in human liver cells [28]. These data indicated that liver progenitor cells could be orientated towards beta-like cell differentiation.

In the pancreas, the ability of alpha cells to differentiate into beta-cells has also been demonstrated in specific experimental rodent models. Indeed, in transgenic mice expressing the receptor for diphtheria toxin (DT-R) in beta cells, it is possible to deplete the mass of beta-cells. Such beta-cell loss, upon receiving an insulin supply, resulted in beta-cell regeneration after 6 months. Experiments tracing cell lineage revealed that alpha cells can transdifferentiate and repopulate the pool of beta cells [29]. This potential of transdifferentiation should now be extended to other models to define its physiological role.

5. Impact of oxygen tension on beta-cell differentiation

Extensive research has focused on the role of transcription factors over the past few years. In our laboratory, we investigated the role of environmental and epigenetic factors that control beta-cell development. Notably, glucose concentration and histone deacetylases were shown to regulate beta-cell differentiation [30–32]. Moreover, oxygen tension was demonstrated to play an important role in pancreas development [33,34]. When undifferentiated rat pancreata were cultured at 3% oxy-
gen (hypoxia), few beta cells developed. In contrast, beta-cell development was enhanced when oxygen tension was increased to 21% (normoxia).

The adaptive responses of cells to hypoxia are mediated by the transcription factor “hypoxia-inducible factor-I-alpha” (HIF1α). Stabilization of HIF depends on the activity of oxygen-dependent prolyl-hydroxylases (PHDs). In normoxia, PHDs are active and hydroxylate two proline residues of HIF1α. Hydroxylation of HIF1α allows its recognition and ubiquitination by the von Hippel-Lindau (E3) ligase complex, leading to the proteosomal degradation of HIF1α. In hypoxia, the activity of PHDs is considerably reduced, allowing HIF1α stabilization. When pancreata were cultured with PHD inhibitors, HIF1α stabilization was forced into normoxia, and beta-cell development was reduced [33,34]. This finding indicates that HIF1α is a repressor of beta-cell differentiation in vitro. During early fetal development, the undifferentiated pancreas expresses HIF1α in the rat and, concordantly, there is a paucity of blood flow [34,35]. Later, blood flow in the pancreas increases, and HIF1α is no longer detected [34,35]. This observation suggests that oxygen tension may play a physiological role in pancreas development.

Other laboratories have investigated the role of HIF1α in mature beta-cells. Genetic invalidation of the von Hippel-Lindau gene (VHL) led to the stabilization of HIF in beta cells. This led to impaired insulin secretion in response to glucose [36–38]. The fact that enhanced vascularization of the transplanted islets favours graft function suggests that HIF1α may also be involved in such a process.

In humans, the HIF complex plays a role in the adaptation to hypoxia, and its expression differs among populations. In the Tibetan population, an allele of *EPAS1*, encoding HIF2α, was found to be associated with low haemoglobin concentration, and was detected at a greatly increased frequency [39]. This event may have resulted from genetic adaptive selection. Furthermore, a functional gain of *EPAS1* was observed in a few individuals/families. However, these subjects were particularly prone to thrombosis and pulmonary hypertension, with increased erythropoiesis due to HIF2α [40]. Thus, we propose that the functional gain of HIF would be disadvantageous in humans. Variants of HIF1α have also been associated with type 1 and type 2 diabetes [41,42]. The parallel between these observations and our findings in the development of the pancreas in rodents suggests that HIF1α may play an important role in the human pancreas. This hypothesis merits further investigation.

6. Conclusion

Recent advances have shed more light on the possibility of islet transplantation in diabetic patients. However, a major limitation remains the poor availability of beta cells. Experimental work in rodents has provided further tools that may help to derive beta cells in vitro. Recent findings concerning the role of oxygen tension in beta-cell development should also help to improve on the current protocols.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgements

The work on oxygen tension was supported by Association de Langue Française pour l’Étude du Diabète et des Maladies Métaboliques (ALFEDIAM; French Language Association for the Study of Diabetes and Metabolic Diseases)/Sanofi-Aventis and the European Foundation for the Study of Diabetes (EFSD)/Novo Nordisk/Juvenile Diabetes Research Foundation International (JDRF). M.H. and A.S. received a fellowship from the French Ministry of Research (ministère de la Recherche).

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