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Revisión

From stem cells to insulin-producing cells: past findings, recent advances and future directions

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Abstract

Restoration of β-cell mass in patients with diabetes is a promising therapeutic option that could alleviate their daily insulin injections and the development of secondary complications. Recent advances in islet implants from cadaveric donors, along with the scarcity of transplantable material, have opened a renewed interest in restoring β-cell function through stem cell bioengineering. There are mainly two stem cell types of great interest for many researchers: embryonic stem cells derived from the inner mass of the developing blastocyst, and adult stem cells present within tissues of the adult organism. Despite the substantial advances in obtaining insulin-producing cells, there are still important questions to be answered before transferring this knowledge to the clinic. These questions include the basic mechanisms operating in the *in vitro* differentiation process, and the necessary conditions to 1) maintain the acquired phenotype, 2) control the proliferation rate, 3) face immune suppression, 4) increase survival of the implanted material, and most importantly, 5) mimic β-cell function. In addition, there is a growing interest in pancreas regeneration, allowing the development of new pharmacological agents that could stimulate β-cell replication and/or neogenesis from pancreatic precursors. The most efficient therapeutic solution is still unknown, but will most likely be a combination of several strategies that adapts to the particular circumstances of each patient. To this end, it is necessary to delve in several fronts in order to solve this complicated puzzle.

Keywords: diabetes, β-cell, stem cells, cell therapy, insulin.

Introduction

Therapeutic approaches towards diabetes must face the multifactorial complexity of the disease. In this context, the simple transfection of the insulin gene into non-pancreatic tissues (gene therapy) only represents a modest advancement that does not guarantee the complete cure of the pathology.[1] Therefore, insulin expression is not the only trait to pursue in regenerative strategies for diabetes treatment. Correct processing of the hormone, glucose sensing and stimulus-secretion coupling are additional key properties to consider when proposing a full therapeutic protocol.[2] The recent, although modest, success in islet transplantation[3-5] has enhanced the interest of several laboratories in many topics related to cell therapy, including embryonic stem cell and adult stem cell reprogramming, pancreas regeneration and β-cell turnover/survival.[6-10]

The progress in areas such as endocrine pancreas ontogenesis, β-cell function and alterations of the diabetic pathology are inspiring new arising concepts in β-cell bioengineering and bioartificial endocrine pancreatic design. Several protocols have been published in this context; however none has been capable of answering key important questions. Compared to adult stem cells (ASCs), embryonic stem cell (ESCs) based protocols have achieved an acceptable degree of differentiation in their final cell products, obtaining cells with hormone contents and several phenotypic traits very similar to those found in adult β-cells.[11] However, obtaining fully functional cells, the risk of tumour formation, immune rejection and implant survival are still unsolved problems in animal models.[2] On the other hand, these questions seem to be less important in ASCs, which display low proliferation rates and no immune rejection provided that donor and acceptor is the same person. However,

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Abbreviations:

ASCs: adult stem cells; BrdU: bromo-deoxyuridine; CK19: cytokeratin-19; EBs: embryoid bodies; ESCs: embryonic stem cells; GIP: glucose-dependent insulinotropic peptide; GLP-1: glucagon-like peptide-1; ZDF rats: Zucker diabetic fatty rats.

protocols to obtain insulin-producing cells derived from ASCs still present modest results, mainly because the operating mechanism (i.e. transdifferentiation, cell fusion, stimulation of pancreatic regeneration) has not been fully elucidated.

In any case, National Granting Agencies must support all possible, and at the same time logical, lines of research. The design of more consistent cell therapy protocols could benefit in the future both type 1 and type 2 diabetic patients. In type 1, the immune system specifically destroys pancreatic β-cells, surpassing their own regenerating potential.[12] For this reason, this disease is the target of many cell therapy-based strategies. Cell substitution protocols or the induction of β-cell replication/neogenesis could be alternatives to consider in advanced stages of type 2 diabetes, mainly when β-cell apoptosis appears.[13,14] This review will intend to summarize the key approaches that have been published in the field of β-cell bioengineering. Their results and conclusions will also be briefly analyzed. Finally, the use of cell therapy and their future perspectives in the battle against diabetes will be evaluated.

Cell therapy strategies based on embryonic stem cells

Our laboratory was the first to demonstrate the proof of principle that mouse ESCs can give rise to insulin-producing cells.[15] In our strategy, mouse R1-ESCs, transfected with the insulin-promoter directing the expression of the neomycin resistance gene, were induced to differentiate. To this end, undifferentiated cells were transferred from attached monolayer cultures to suspension cultures, forming cell aggregates called embryoid bodies (EBs). EBs are cell clusters that favour cell differentiation processes by still unknown mechanisms. Once this step was achieved, cells were allowed to attach again (outgrowth phase) and insulin$^+$-cells were selected by adding neomycin to the culture medium. Therefore, the presence of insulin$^+$ cells as the final product was based on the spontaneous expression of the insulin-gene. However, the final cell product was actually formed by a heterogeneous population of cells, deriving from the neuroectoderm (most abundant), primitive endoderm and definitive endoderm. In addition, in this report we advanced the key role of several determinants, such as the incubation in the presence of nicotinamide and low glucose concentration in the outgrowth phase, as well as cell cluster formation before transplantation into the spleen of streptozotocin-diabetic mice. Interestingly, similar results were subsequently reported in human ESCs.[16] However, the variable amounts of insulin produced by the different clones constrained the reproducibility of the protocol. Only clones derived from initial passages of R1-ESCs resulted in euglycaemia recovery in transplanted animals. Late passages of R1-ESCs produced lower amounts of insulin (mainly insulin II), indicating a clear commitment to neuroectoderm-derived insulin$^+$ cells with a major risk of teratoma formation in transplanted animals.[17,18] Altogether, these observations suggest a redefinition of the strategy by taking in account two points: A) The necessity of well-designed coaxial strategies in order to increase reproducibility and B) To initially commit the cells to definitive endoderm, the embryonic layer from which the endocrine pancreas derives, by assessing the expression of preproinsulin I (the main gene product of pancreatic β-cells).

Concerning coaxial strategies, Lumelsky and coworkers proposed a protocol widely used in many laboratories.[19] The strategy was based on the idea that nestin$^+$ neurons and pancreatic β-cells share many features, allowing the possibility of obtaining insulin$^+$-cells from ectoderm-derived cells. Indeed, specific transcription factors, proteins of the glucose-sensing machinery and components of the secretory pathway are well expressed in both hypothalamic neurons and pancreatic β-cells.[20-22] However, from a phenotypic and functional point of view, neurons and β-cells markedly differ, as it has been clearly evidenced in this group of protocols.[23, 24] The cells obtained using this strategy only expressed the insulin II gene, which is barely expressed in mouse pancreatic islets.[25] Also, there was no information concerning the hormone processing, and the euglycaemia recovery experiments in transplanted animals were not fully convincing.[26] In this sense, teratoma formation could be observed after implantation[19] or, in the best of the cases, a recovery similar to that seen in animals transplanted with non-differentiated cells.[27] We analyzed in more detail these tumour cells by tracing them with specific gene markers. As a result, the cells were still capable of expressing high levels of pluripotential markers (Oct3/4, Nanog and Esg-1) and histone H2AX, possessed an altered pattern of c-myc phosphorylation, aberrant chromosomal numbers (trisomy of chromosomes 8 and 9) and a high degree of BrdU (bromodeoxyuridine) incorporation.[18,28] Altogether, these observations indicate that nestin-based selection

protocols still require substantial improvements in order to obtain functional cell products that are similar to mature β-cells.

As we predicted in a previous publication,[17] to circumvent the limitations posed by neuroectodermal derivatives it is necessary to initially commit the cells to definitive endoderm. Although this idea was repetitively developed by our group in several grant proposals to the Ministry of Science during 4 years and reiteratively annulled, it was a laboratory in San Diego which finally designed a protocol prototype.[11] The rationale was to enrich the human ESC monolayers in definitive endoderm precursors and then to incubate these cells following 5 steps, imitating the key steps in pancreas ontogenesis (figure 1). Of great importance in this protocol was the fact that the resulting cells possessed the highest insulin content described so far, similar to the amounts found in adult β-cells. However, the authors claimed that the insulin$^+$ cells did not display a mature functional phenotype.

In any case, this protocol has settled an important tool for future research, opening new questions and redefining old dogmas. From the obtained results, it is clear that it is necessary to delve in human pancreas development, but at the same time more information must be acquired concerning β-cell function in normal and in diabetic conditions. Since the protocol only works with definitive endoderm committed cells, but not with neuroectoderm or visceral endoderm, the pathway to obtain *in vitro* definitive endoderm has to be clearly defined and specific markers need to be detected.[29,30] In this context, *in vitro* experiments seem to suggest that definitive endoderm could derive from a progenitor mesendodermic cell population, which as its name suggests is capable of giving rise to both mesoderm and endoderm fates.[31] In this context, Brachyury (a mesendoderm marker)-selected cells can give rise to modest amounts of insulin I$^+$ cells, confirming the previous hypothesis.[32] The addition of activin A and serum deprivation seem to improve the yield of cells committed to definitive endoderm from mesendodermic precursors in human ESC cultures.[29,30]

Several additional questions remain to be addressed in Baetge's protocol[11] in order to transfer this technology to a more therapeutic context. First, the differentiation protocol presents an artificial situation in which one day of the *in vitro* protocol recapitulates what occurs in one week of normal *in vivo* pancreas development. The restricted culture conditions in terms of space in the culture dish could favour differentiation processes over proliferation, which usually has longer periods of time during mammalian organogenesis. Several aspects need to be solved concerning the resulting immature cells. In this sense, the final cell product bears unprocessed proinsulin, low secretory response to extracellular glucose concentrations and co-expression of insulin with other hormones, such as glucagon or somatostatin, in the same cell. In this sense, cell tracing experiments using transgenic models unequivocally indicate that pancreatic α and β-cells arise from independent lineages, at least in

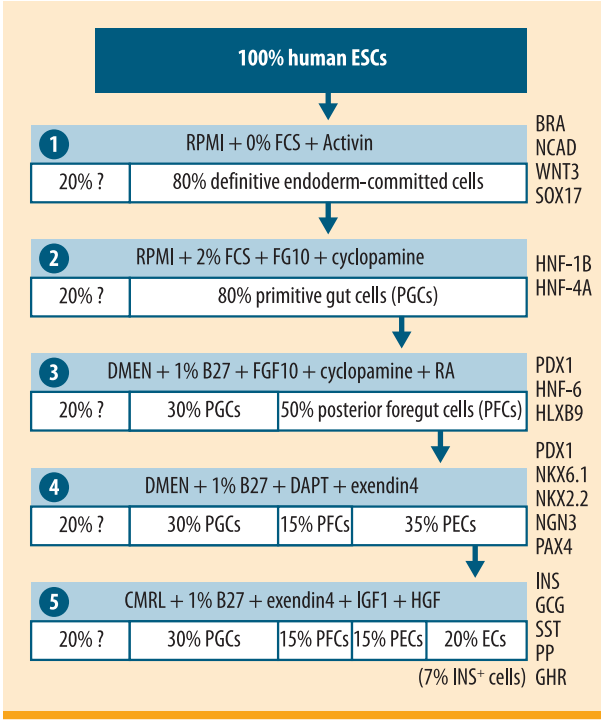

Figure 1. Protocol to differentiate human ESCs to insulin-producing cells developed by Dr Emmanuel Baetge (San Diego, USA)[11]. The differentiation protocol is divided in 5 stages in which different growth factors, chemicals, medium and serum (FBS) are added. Stage 1: Definitive endoderm, Stage 2: Primitive gut tube, Stage 3: Posterior foregut, Stage 4: Pancreatic endoderm and Stage 5: Pancreatic endocrine cells. Markers for each stage are listed in capital letters on the right. The percentages of cells obtained in each condition are indicated. This protocol lasts approximately 2-3 weeks, resulting in 7% of insulin$^+$ cells at the end of stage 5. In this stage, the cells also express NEUROD1, ISL1, PAX6, SYP (synaptophysin) and IAPP (islet amyloid polypeptide). Other abbreviations used: BRA: brachyury, DAPT: γ-secretase inhibitor, ECs: endocrine cells, FGF10: fibroblast growth factor-10, GHR: ghrelin, GCG: glucagon, HGF: hepatocyte growth factor, IGF1: insulin-like growth factor-1, INS: insulin, NCAD: N-cadherin, PECs: pancreatic endocrine-precursor cells, PP: pancreatic polypeptide, RA: all-trans retinoic acid, SST: somatostatin, ?: cells of unknown origin

rodents.[33] However, this and other *in vitro* protocols very often present cells co-expressing both hormones.[34] In addition, glucagon and insulin are very often co-expressed in insulinoma cell lines. In particular, PDX-1 downregulation, which subsequently decreases insulin gene expression, favours preproglucagon expression in tumour cell lines.[35] Gene expression artefacts due to particular culture conditions cannot be discarded either. Additionally, the expression of transcription factor MAFA, which is required for the progression to a mature β-cell phenotype,[36] has not been reported in this strategy.[11] Finally, this protocol seems to be restricted to certain cell lines. In this sense, there is no explanation to why H7 and H9 human ESCs can be successfully committed to definitive endoderm,[29] but fail to derive to pancreatic precursors.[11] These and other questions need to be answered in order to design a more universal protocol for β-cell differentiation.

The classical approach via EB formation allows spontaneous expression of preproglucagon gene, suggesting differentiation to definitive endoderm and indicating that part of the pathway described by Baetge could be spontaneously recapitulated in EBs structures. However, glucagon$^+$-cells obtained in these protocols have not been characterized in detail in order to establish its real origin. Indeed, glucagon is well expressed in endoderm derived tissues, such as endocrine pancreatic α-cells and intestinal L-cells, as well as in the neuroectoderm derived hypothalamus, thalamus and septal regions of the brain.[35] Nevertheless, the final product differs in these cell types: preproglucagon in α-cells and glucagon-like peptide-1 (GLP-1) in L-cells and neuroectoderm derived tissues.[37] Therefore, we cannot ascribe that 100% of glucagon$^+$-cells found in EBs are derivatives from the definitive endoderm, since a neuroectodermic origin of glucagon$^+$-cells in bioengineering protocols cannot be discarded.

The unsolved aspects of Baetge's protocol open the possibility of searching for new determinants to complete the differentiation process. When human ESCs were co-transplanted with mouse foetal dorsal pancreas, they began to express processed insulin as well as many pancreatic transcription factors,[38] suggesting that in the niche where pancreas develops there should exist a source to fish the key differentiation factors that could complement this protocol. This was the rationale behind a granted project in which transfected mouse D3-ESCs with the insulin promoter-neomycin resistance gene were incubated in the presence of low serum and conditioned media from pancreatic rudiment cultures of embryonic day –16.5.[39] Incomprehensibly, this study did not report the real origin of the obtained insulin$^+$-cells. Similar experiments performed by our group revealed that only insulin II gene was expressed, along with Amnionless, indicating that this was an excellent protocol to obtain primitive endoderm but not definitive endoderm (figure 2). This was confirmed by the lack of expression of the glucagon gene, which was not reported in the original publication. Data concerning the processing of insulin were also obviated. The release of 20% of the insulin content when final cells were stimulated with glucose is very unusual and far from what is observed in mature β-cells. Finally, the large amounts of BrdU incorporated by the cells strongly indicated that the final

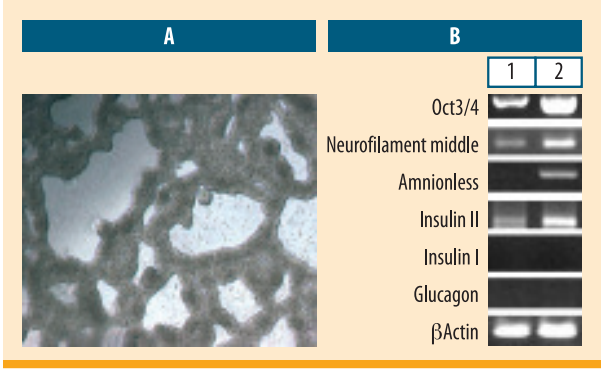

Figure 2. EB formation (A) and gene expression pattern (B) of insulin$^+$ cells obtained according to the protocol developed in reference 39. Mouse D3-ESCs were transfected with a DNA construct containing the human insulin promoter directing the expression of the neomycin-resistance gene. The differentiation protocol commenced by EB formation in non-adherent plates at 3% foetal calf serum (FCS) in absence of LIF and in the presence of pancreatic bud conditioned medium for 7d. Cells were transferred to adherent plates under the same culture conditions but with 10% FCS instead and neomycin selection was performed. The rationale was to use the pancreatic soluble factors released by the buds which would direct the differentiation process. A) First step of directed differentiation: Resulting EBs (1.5 x 10^6 cells/mL) (see page 259 of reference 39) according to the protocol published after hygromycin selection. Bar: 100 µm. B) Gene expression pattern analysis by RT-PCR of undifferentiated mouse D3-cells (1) and obtained cells in the outgrowth phase at the end of the protocol (2). Final cells were positive for Neurofilament middle (neuroectoderm marker), Amnionless (primitive endoderm marker), Oct3/4 (undifferentiated cells and neuroectoderm marker) and insulin II (neuroectoderm and primitive endoderm marker). No expression of insulin I and glucagon genes (definitive endoderm markers, the embryo layer from which β-cell derives) was observed. Same results were obtained with a new batch of D3-cells (purchased from ATTC, Middlesex, UK), EBs formed with 25 x 10^3 cells/mL and no neomycin selection

cell obtained could produce tumours in transplanted animals, point which was elegantly bypassed by the authors by simply sacrificing the animals after 15 d post-implantation.

In any case, a new design of this approach could be exploited in a more rational manner to search for extracellular factors that could complement an *in vitro* universal protocol, obtaining functional β-cells amenable for transplantation trials. To this end, it is necessary to discover the unknown aspects of β-cell physiology in the adult and developing organism.[40,41]

Cell therapy strategies based on adult stem cells

The low proliferation rate, which diminishes the risk of tumour formation, along with its immune compatibility, make ASCs an interesting alternative to consider in cell therapy strategies for diabetes. In this context, it is important to discriminate between pancreatic and extrapancreatic stem cells (figure 3). The search for pancreatic progenitors has been hampered by the observation that

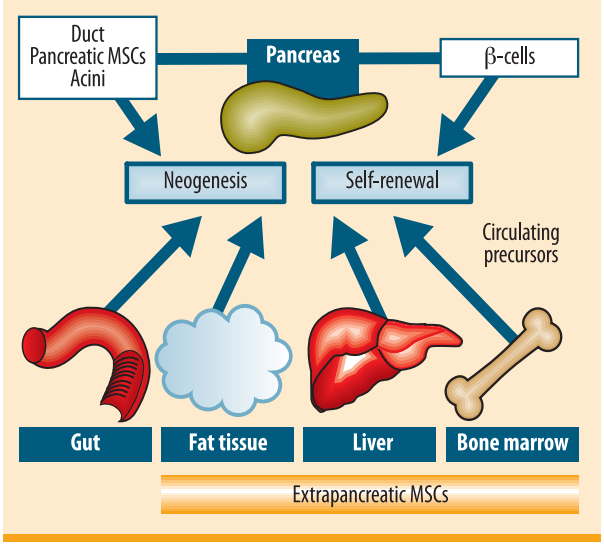

Figure 3. Possibilities offered by ASCs in diabetes cell therapy. Several pancreatic cells could contribute to β-cell regeneration: β-cell self-replicating mechanisms, or ductal, acinar or mesenchymal cells by giving rise to new β-cells (neogenesis). Other extrapancreatic tissues could contribute to β-cell regeneration by neogenesis as well as through self-renewal mechanisms. Neogenesis could be achieved from tissue specific stem cells or extrapancreatic mesenchymal stem cells (MSCs) by transdifferentiation and self-renewal, providing extracellular factors that could enhance β-cell division. Circulating factors should be also taken into account

the pancreas does not regenerate when it suffers partial ablation, conversely to other endoderm-derived organs, such as the liver. Despite the large amount of research being performed, the location and identification of a pancreatic stem cell population remains elusive. Several candidates have been proposed, including ductal cells, exocrine-associated cells, pancreatic mesenchymal cells as well as the β-cells themselves.

The observation of islet budding from pancreatic ducts has suggested the existence of islet precursors in this structure. Budding can be stimulated by certain factors such as INGAP (Islet Neogenesis-Associated Protein) peptide or all-trans retinoic acid.[42,43] These candidates have been given several names, such as CHIBs (Cultivated Human Islet Buds) expressing cytokeratin-19 (CK19),[44] IPSCs (Islet Pluripotent Stem Cells),[45] NIPs (Nestin-positive Islet-derived Progenitors)[46] and NEPEC (Non-Endocrine Pancreatic Epithelial Precursor).[47] NIPs, which express nestin, have been found to be also associated with islet structures and have been identified as most likely being pancreatic mesenchymal stem cells.[48] Recently, Neurogenin3^{+} cells have been described as β-cell precursors.[49] At least *in vitro*, insulin^{+}-cells derived from ductal precursors produce modest amounts of insulin, and their stimulus-coupled secretory response is very poor, requiring further improvements. On the other hand, endocrine pancreatic precursors have been described in the exocrine portion, though the true nature of this population remains unknown.[50,51]

Finally, it seems that new β-cells can arise from replication processes of pre-existing β-cells.[52] Convincing cell tracing experiments have demonstrated this point, but the limitation of this experimental approach does not give any clues concerning the nature of the pancreatic progenitors, which most likely do not express insulin. In any case, β-cell turnover could be the standard mechanism to renew these endocrine cells during the life cycle as well as in pregnancy, and most likely are activated during the first stages of type 2 diabetes, which is characterised by the presence of insulin resistance.[8,53] However, when overt diabetes, either type 1 or type 2, is declared, accelerated β-cell destruction occurs, at a rate that the self-regeneration mechanisms cannot compensate the amount of β-cell lost.[52,54] An interesting question that has not been properly addressed in the current studies is how this unfavourable environment can also hamper the pancreatic progenitor's function. At present, little

is known about the antigen determinants displayed by these progenitors or their sensitivity to cytokines which could prone these cells to suffer similar damage processes as mature β-cells in type 1 diabetes. This rationale can also be applied to type 2 diabetes, where self-suicide mechanisms could be activated in the precursor cell population. This idea is supported by the observation of triglyceride accumulation not only in β-cells, but in pancreatic fibroblasts as well of ZDF (Zucker diabetic fatty) rats, suggesting that lipotoxic mechanisms could affect this cell population.[14]

On the other hand, the possibility of bioengineering extrapancreatic ASCs to obtain insulin-producing cells is supported by the accumulated experience of ectopic insulin expression in gene therapy protocols.[1,55,56] In this sense, bone marrow represents an important ASC population in which its transdifferentiation to insulin-secreting cells has been proven,[57] although it has not been reproduced by others.[58-62] The explanation could be that bone marrow harbours a heterogeneous population of progenitors, including haematopoietic, endothelial, preadipoblast and mesenchymal progenitors, among others, bearing different degrees of plasticity and thereby culminating in distinct differentiation processes with a very diverse efficiency. In addition, insulin$^+$-cell self-renewal from bone marrow ASCs cannot dwell exclusively in transdifferentiation mechanisms. Other possibilities, such as cell fusion and angiogenesis, could participate in this process in different degrees.[63,64]

A key conclusion raised is that a precise phenotyping of precursors would be mandatory in all published reports in order to avoid problems related with reproducibility when these experiments are carried out in other laboratories. For instance, a population derived from circulating human monocytes could be coaxed to express the insulin gene and possess very modest amounts of C-peptide.[65] Several authors of this work publicly claimed in scientific forums the difficulty to reproduce these results. The lack of a precise phenotypic characterization of these precursors could explain this arbitrary variability. In this sense, insulin$^+$ cells were obtained in another laboratory from a human stem cell population derived from peripheral blood. The phenotypic characterization showed that these cells were positive for Oct-4, Nanog and the haematopoietic markers CD9, CD45 and CD117, while negative for CD34 and markers of the monocyte or lymphocyte lineages.[66]

The liver derives from the upper foregut definitive endoderm, similar to the pancreas; however, this organ displays a robust self-renewing capacity. In this context, oval hepatic stem cells are precursors capable of differentiating into hepatocytes, bile duct epithelium and also to insulin-positive cells.[67,68] In addition, the over-expression of the pancreatic transcription factor PDX-1 allows the transdifferentiation of human foetal hepatic stem cells[69] and adult human and mouse hepatocytes into insulin-producing cells.[70,71] However, PDX-1 could additionally induce the differentiation to pancreatic exocrine tissue, causing hepatic destruction. Transfection with more specific transcription factors of the endocrine pancreas (i.e. NeuroD) combined with betacellulin, gave rise to insulin$^+$-cells with no such secondary effects.[72]

Following the same rationale as in liver, endoderm-derived endocrine cells of the intestinal epithelium might be good candidates for ectopic insulin expression and secretion. K-enteroendocrine cells share certain functional traits with pancreatic β-cells, such as glucokinase expression, the hormone processing machinery and glucose-dependent secretion of the glucose-dependent insulinotropic peptide (GIP). Transfection of a tumour-derived K-cell line with the human insulin gene under control of the GIP-promoter resulted in glucose regulated hormone secretion in a very similar fashion to that observed in certain β-cell lines.[73] However, the transfer of such technology to a real *in vivo* situation still requires important amendments.

Finally, mesenchymal stem cells, which are present in many organs including the pancreas, represent another alternative source to obtain insulin-secreting cells. Although our knowledge is limited, it seems that these cells represent a heterogeneous population inside the organ.[74,75] It has been proposed that in the pancreas, insulin-producing cells may arise by a mechanism called the epithelial-to-mesenchymal-to-epithelial transition which implies the dedifferentiation of islet cells to mesenchymal cells in order to re-differentiate again into new islets.[76,77] However, whether this mechanism, which has only been described *in vitro*, also occurs *in vivo* is a debated question.[78-82] On the other hand, mesenchymal cells from the bone marrow stroma, adipose tissue and liver can give rise to insulin-producing cells through the use of different reprogramming strategies.[83-87] However, data from functional secretion tests and glycaemia management in transplanted animal models are still lacking.

Table 1. Surface expression profile of mesenchymal stem cell (MSCs) clones isolated from human liver (L) or bone marrow (BM) that can give rise to insulin$^+$ cells

	L-MSCs	BM-MSCs		
Antigen	(88)	(85)	(89)	(90)
CD9				+
CD10				+
CD11		–		
CD13				+
CD14			–	
CD29	+		+	+
CD31		–		
CD34[a]	–	–	–	–
CD44	+	+	+	+
CD45[a]	–	–	–	–
CD49		–		
CD73	+	+		+
CD90	+	+		+
CD105		+		+
CD106			+	
CD117	–	–		
CD133	–			
CD147		+		
CD166				+

[a]Hematopoietic markers. References are indicated between brackets.

In any case, it is important to note that mesenchymal cell cloning (table 1) can substantially increase the reproducibility of the results *in vitro*, obtaining productive cells in terms of hormone biosynthesis and regulated secretion.

Conclusions

Research on ESCs and ASCs as potential sources for the treatment of diabetes requires substantial improvements before transferring this technology to a clinical context. In both cases, *in vitro* culture conditions need to be fine-tuned in order to establish a universal protocol, which means reproducibility in any laboratory and not dependent of the cell line used. Parallel advances in ESC and ASC bioengineering will improve the customization of the final cell product which must intend to be functional in terms of insulin processing and secretion, immunocompatible and free of exacerbated proliferation or limited survival. Research in pancreatic ASCs must also be strongly supported, since this will allow the optimization of pancreas donations through the stimulation of islet replication or islet neogeneis in order to increase the quality of the transplantable biomass. In addition, novel pharmacological agents that could stimulate *in vivo* islet/β-cell turnover and/or neogenesis need to be developed.

To achieve this enormous task, it is necessary to continue investigating the mechanisms underlying endocrine pancreas pathophysiology and development. In this context, National Founding Organisms must support innovative ideas that could represent significant advances in this field rather than support projects coming from laboratories that can only provide an image or, even worst, irreproducible results. The follow-up of the projects must contemplate the same rigor and exigency as the initial evaluation. To this end, reviewers must first have certain knowledge in the field, and be ethical in their judgements, letting aside political and economical benefits. Only in this context, we can fulfil the ancient Bible phrase: "Render to Caesar the things that are Caesar's, and to God the things that are God's".

Acknowledgements

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References

1. Efrat S. Prospects for gene therapy of insulin-dependent diabetes mellitus. Diabetologia. 1998;41:1401-9.
2. Roche E, Reig JA, Campos A, Paredes B, Isaac JR, Lim S, et al. Insulin-secreting cells derived from stem cells: Clinical perspectives, hypes and hopes. Transplant Immunol. 2005;15:113-29.
3. Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med. 2000; 343:230-8.
4. Ryan EA, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, Shapiro AM. Five-year follow-up after clinical islet transplantation. Diabetes. 2005;54:2060-9.
5. Hogan A, Pileggi A, Ricordi C. Transplantation: current developments and future directions; the future of clinical islet transplantation as a cure for diabetes. Front Biosci. 2008;13:1192-205.
6. Madsen OD, Serup P. Towards cell therapy for diabetes. Nat Biotech. 2006; 24:1481-3.
7. Fellous TG, Guppy NJ, Brittan M, Alison MR. Cellular pathways to β-cell replacement. Diabetes Metab Res Rev. 2007;23:87-99.
8. Murtaugh LC. Pancreas and beta-cell development: from the actual to the possible. Development. 2007;134:427-438.

9. Lee SC, Pervaiz S. Apoptosis in the pathophysiology of diabetes mellitus. Int J Biochem Cell Biol. 2007;39:497-504.
10. Hochedlinger K, Jaenisch R. Nuclear reprogramming and pluripotency. Nature. 2006;441:1061-7.
11. D'Amour KA, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG, et al. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotech. 2006;24:1392-401.
12. Isermann B, Ritzel R, Zorn M, Schilling T, Nawroth PP. Autoantibodies in diabetes mellitus: current utility and perspectives. Exp Clin Endocrinol Diabetes. 2007;115:483-90.
13. Butler PC, Meier JJ, Butler AE, Bhushan A. The replication of beta cells in normal physiology, in disease, and for therapy. Nat Clin Pract Endocrinol Metab. 2007;3:758-68.
14. Prentki M, Nolan CJ. Islet β cell failure in type 2 diabetes. J Clin Invest. 2006;116:1802-12.
15. Soria B, Roche E, Bernat G, Leon-Quinto T, Reig JA, Martin F. Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice. Diabetes. 2000;49:157-62.
16. Assady S, Maor G, Amit M, Itskovitz-Eldor J, Skorecki KL, Tzukerman M. Insulin production by human embryonic stem cells. Diabetes. 2001;50:1691-7.
17. Roche E, Sepulcre P, Reig JA, Santana A, Soria B. Ectodermal commitment of insulin-producing cells derived from mouse embryonic stem cells. FASEB J. 2005;19:1341-3.
18. Ensenat-Waser R, Santana A, Vicente-Salar N, Cigudosa JC, Roche E, Soria B, et al. Isolation and characterization of residual undifferentiated mouse embryonic stem cells from embryoid body cultures by fluorescence tracking. *In Vitro* Cell Develop Biol-Animal. 2006;42:115-23.
19. Lumelsky N, Blondel O, Laeng P, Velasco I, Ravin R, McKay R. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. Science. 2001;292:1389-94.
20. Yang XJ, Kow LM, Funabashi T, Mobbs CHV. Hypothalamic glucose sensor. Similarities to and differences from pancreatic β-cell mechanisms. Diabetes. 1999;48:1767-72.
21. Schuit FC, Huypens P, Heimberg H, Pipeleers DG. Glucose sensing in pancreatic beta-cells: a model for the study of other glucose-regulated cells in gut, pancreas, and hypothalamus. Diabetes. 2001;50:1-11.
22. Gerber SH, Südhof TC. Molecular determinants of regulated exocytosis. Diabetes. 2002;51 (Suppl 1):S3-S11.
23. Miyazaki S, Yamato E, Miyazaki J. Regulated expression of Pdx-1 promotes *in vitro* differentiation of insulin-producing cells from embryonic stem cells. Diabetes. 2004;53:1030-7.
24. Moritoh Y, Yamato E, Yasui Y, Miyazaki S, Miyazaki J. Analysis of insulin-producing cells during *in vitro* differentiation from feeder-free embryonic stem cells. Diabetes. 2003;52:1163-8.
25. Melloul D, Marshak S, Cerasi E. Regulation of insulin gene transcription. Diabetologia. 2002;45:309-26.
26. Rajagopal J, Anderson WJ, Kume S, Martinez OI, Melton DA. Insulin staining of ES cell progeny from insulin uptake. Science. 2003;299:363.
27. Blyszczuk P, Czyz J, Kania G, Wagner M, Roll U, St-Onge L, et al. Expression of Pax4 in embryonic stem cells promotes differentiation of nestin-positive progenitor and insulin-producing cells. Proc Natl Acad Sci USA. 2003;100:998-1003.
28. Santana A, Enseñat-Waser R, Arribas MI, Reig JA, Roche E. Insulin-producing cells derived from stem cells: recent progress and future directions. J Cell Mol Med. 2006;10:866-83.
29. D'Amour KA, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE. Efficient differentiation of human embryonic stem cells to definitive endoderm. Nat Biotech. 2005;23:1534-1541.
30. Yasunaga M, Tada S, Torikai-Nishikawa S, Nakano Y, Okada M, Jakt LM, et al. Induction and monitoring of definitive and visceral endoderm differentiation of mouse ES cells. Nat Biotech. 2005;23:1542-50.
31. Tada S, Era T, Furusawa C, Sakurai H, Nishikawa S, Kinoshita M, et al. Characterization of mesendoderm: a diverging point of the definitive endoderm and mesoderm in embryonic stem cell differentiation culture. Development. 2005;132:4363-74.
32. Kubo A, Shinozaki K, Shannon JM, Kouskoff V, Kennedy M, Woo S, et al. Development of definitive endoderm from embryonic stem cells in culture. Development. 2004;131:1651-62.
33. Herrera PL. Defining the cell lineages of the islets of Langerhans using transgenic mice. Int J Dev Biol. 2002;46:97-103.
34. Segev H, Fishman B, Ziskind A, Shulman M, Itskovitz-Eldor J. Differentiation of human embryonic stem cells into insulin-producing clusters. Stem Cells. 2004;22:265-74.
35. Gromada J, Franklin I, Wollheim CB. Alpha-cells of the endocrine pancreas: 35 years of research but the enigma remains. Endocr Rev. 2007;38:84-116.
36. Kaneto H, Miyatsuka T, Fujitani Y, Noguchi H, Sang KH, Yoon KH, et al. Role of PDX-1 and MafA as a potential therapeutic target for diabetes. Diabetes Res Clin Pract. 2007;77 (Suppl 1):S127-S137.
37. Kieffer TJ, Habener JF. The glucagon-like peptides. Endocr Rev. 1999;20:876-913.
38. Brolén GKC, Heins N, Edsbagge J, Semb H. Signals from the embryonic mouse pancreas induce differentiation of human embryonic stem cells into insulin-producing β-cell-like cells. Diabetes. 2005;54:2867-74.
39. Vaca P, Martin F, Vegara-Meseguer JM, Rovira JM, Bernat G, Soria B. Induction of differentiation of embryonic stem cells into insulin secreting cells by fetal soluble factors. Stem Cells. 2006;24:258-65.
40. Martin M, Hauer V, Messmer M, Orvain C, Gradwohl G. Transcription factors in pancreatic development. Animal models. Endocr Dev. 2007;12:24-32.
41. Scharfmann R. Pancreatic development as a basis for the definition of new therapies for diabetes. Endocr Dev. 2007;12:1-11.
42. Roche E, Jones J, Arribas MI, Leon-Quinto T, Soria B. Role of small bioorganic molecules in stem cell differentiation to insulin-producing cells. Bioorg Med Chem. 2006;14:6466-74.
43. Rafaeloff R, Pittenger GL, Barlow SW, Qin XF, Yan B, Rosemberg L, et al. Cloning and sequencing of the pancreatic islet neogenesis associated protein (INGAP) gene and its expression in islet neogenesis in hamsters. J Clin Invest. 1997;99:2100-9.
44. Bonner-Weir S, Taneja M, Weir GC, Tatarkiewicz K, Song KH, Sharma A, et al. *In vitro* cultivation of human islets from expanded ductal tissue. Proc Natl Acad Sci USA. 2000;97:7999-8004.
45. Ramiya VK, Maraist M, Arfors KE, Schatz DA, Peck AB, Cornelius JG. Reversal of insulin-dependent diabetes using islets generated *in vitro* from pancreatic stem cells. Nat Med. 2000;6:278-82.
46. Zulewski H, Abraham EJ, Gerlach MJ, Daniel PB, Moritz W, Müller B, et al. Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine and hepatic phenotypes. Diabetes. 2001;50:521-33.
47. Hao E, Tyrberg B, Itkin-Ansari P, Lakey JR, Geron I, Monosov EZ, et al. Beta-cell differentiation from nonendocrine epithelial cells of the adult human pancreas. Nat Med. 2006;12:310-6.
48. Zulewski H. Stem cells with potential to generate insulin-producing cells in man. Swiss Med Wkly. 2006;136:647-54.
49. Xu X, D'Hoker J, Stangé G, Bonné S, De Leu S, Xiao X, et al. β Cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell. 2008;132:197-207.
50. Minami K, Okuno M, Miyawaki K, Okumachi A, Ishizaki K, Oyama K, et al. Lineage tracing and characterization of insulin-secreting cells generated from adult pancreatic acinar cells. Proc Natl Acad Sci USA. 2005;102:15116-21.
51. Desai BM, Oliver-Krasinski J, De Leon DD, Fazard C, Hong N, Leach SD, et al. Preexisting pancreatic acinar cells contribute to acinar cell, but not islet beta cell regeneration. J Clin Invest. 2007;117:971-7.
52. Dor Y, Brown J, Martínez OI, Melton DA. Adult pancreatic β-cells are formed by self-duplication rather than stem-cell differentiation. Nature. 2004;429:41-6.

53. Ackermann AM, Gannon M. Molecular regulation of pancreatic β-cell mass development, maintenance, and expansion. J Mol Endocrinol. 2007;38:193-206.
54. Kodama S, Kuhtreiber W, Fujimura S, Dale EA, Faurtman DL. Islet regeneration during the reversal of autoimmune diabetes in NOD mice. Science. 2003;302:1223-7.
55. Zaia JA. The status of gene vectors for the treatment of diabetes. Cell Biochem Biophys. 2007;48:183-190.
56. D'Anneo A, Rood P, Bottino R, Balamurugan AN, He J, Glannoukakis N. Gene therapy for type 1 diabetes: is it ready for the clinic? Immunol Res. 2006;36:83-9.
57. Ianus A, Holz GG, Theise ND, Hussain MA. *In vivo* derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion. J Clin Invest. 2003;111:843-50.
58. Choi JB, Uchino H, Azuma K, Iwashita N, Tanaka Y, Mochizuki H, et al. Little evidence of transdifferentiation of bone marrow-derived cells into pancreatic beta cells. Diabetologia. 2003;46:1366-74.
59. Lechner A, Yang Y-Q, Blacken RA, Wang L, Nolan AL, Habener JF. No evidence for significant transdifferentiation of bone marrow into pancreatic β-cells *in vivo*. Diabetes. 2004;53:616-23.
60. Hasegawa Y, Ogihara T, Yamada T, Ishigaki Y, Imai J, Uno K, et al. Bone marrow (BM) transplantation promotes beta-cell regeneration after acute injury through BM cell mobilization. Endocrinology. 2007;148:2006-15.
61. Butler AE, Huang A, Rao PN, Bhushan A, Hogan WJ, Rizza RA, et al. Hematopoietic stem cells derived from adult donors are not a source of pancreatic beta-cells in adult nondiabetic humans. Diabetes. 2007;56:1810-16.
62. Lavazais E, Pogu S, Saï P, Martignat L. Cytokine mobilization of bone marrow cells and pancreatic lesion do not improve streptozotocin-induced diabetes in mice by transdifefrentiation of bone marrow cells into insulin-producing cells. Diabetes Metab. 2007;33:68-78.
63. Hess D, Li L, Martin M, Sakano S, Hill D, Strutt B, et al. Bone marrow-derived stem cells initiate pancreatic regeneration. Nat Biotech. 2003;21:763-70.
64. Voltarelli JC, Couri CEB, Stracieri ABPL, Oliveira MC, Moraes DA, Pieroni F, et al. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. JAMA. 2007;297:1568-76.
65. Ruhnke M, Ungefroren H, Nussler A, Martin F, Brulport M, Schormann W,et al. Differentiation of *in vitro*-modified human peripheral blood monocytes into hepatocyte-like and pancreatic islet-like cells. Gastroenterology. 2005;128:1774-86.
66. Zhao Y, Huang Z, Lazzarini P, Wang Y, Di A, Chen M. A unique human blood-derived cell population displays high potencial for producing insulin. Biochem Biophys Res Commun. 2007;360:205-11.
67. Yang L, Li S, Hatch H, Ahrens K, Cornelius JG, Petersen BE, et al. *In vitro* trans-differentiation of adult hepatic stem cells into pancreatic endocrine hormone-producing cells. Proc Natl Acad Sci USA. 2002;99:8078-83.
68. Kim S, Shin JS, Kim HJ, Fisher RC, Lee MJ, Kim CW. Streptozotocin-induced diabetes can be reversed by hepatic oval cell activation through hepatic transdifferentiation and pancreatic islet regeneration. Lab Invest. 2007;87:702-12.
69. Zalzman M, Gupta S, Giri RK, Berkovich I, Sappal BS, Karnieli O, et al. Reversal of hyperglycemia in mice by using human expandable insulin-producing cells differentiated from fetal liver progenitor cells. Proc Natl Acad Sci USA. 2003;100:7253-8.
70. Ferber S, Halkin A, Cohen H, Ber I, Einav Y, Goldberg I, et al. Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. Nat Med. 2000;6:568-72.
71. Shternhall-Ron K, Quintana FJ, Perl S, Meivar-Levy I, Barshack I, Cohen IR, et al. Ectopic PDX-1 expression in liver ameliorates type 1 diabetes. J Autoimmun. 2007;28:134-42.
72. Kojima H, Fujimiya M, Matsumura K, Younan P, Imaeda H, Maeda M, et al. NeuroD-betacellulin gene therapy induces islet neogenesis in the liver and reverses diabetes in mice. Nat Med. 2003;9:596-603.
73. Cheung AT, Dayanandan B, Lewis JT, Korbutt GS, Rajotte RV, Bryer-Ash M, et al. Glucose-dependent insulin release from genetically engineered K cells. Science. 2000;290; 1959-62.
74. Jackson L, Jones DR, Scotting P, Sottile V. Adult mesenchymal stem cells: Differentiation potential and therapeutic applications. J Postgrad Med. 2007;53:121-7.
75. Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: Mesenchymal stem cells: Their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells. 2007;25:2739-49.
76. Gershengorn MC, Hardikar AA, Wei C, Geras-Raaka E, Marcus-Samuels B, Raaka BM. Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells. Science. 2004;306:2261-4.
77. Davani B, Ikonomou L, Raaka BM, Geras-Raaka E, Morton RA, Marcus-Samuels B, et al. Human islet-derived precursor cells are mesenchymal stromal cells that differentiate and mature to hormone-expressing cells *in vivo*. Stem Cells. 2007;25:3215-22.
78. Atouf F, Park CH, Pechhold K, Ta M, Choi Y, Lumelsky NL. No evidence for mouse pancreatic beta-cell epithelial-mesenchymal transition *in vitro*. Diabetes. 2007;56:699-702.
79. Chase LG, Ulloa-Montoya F, Kidder BL, Verfaillie CM. Islet-derived fibroblast-like cells are not derived via epithelial-mesenchymal transition from Pdx-1 or insulin-positive cells. Diabetes. 2007;56:3-7.
80. Eberhardt M, Salmon P, von Mach MA, Hengstler JG, Brulport M, Linscheid P, et al. Multipotential nestin and Isl-1 positive mesenchymal stem cells isolated from human pancreatic islets. Biochem Biophys Res Commun. 2006;345:1167-76.
81. Weinberg N, Ouziel-Yahalom L, Knoller S, Efrat S, Dor Y. Lineage tracing evidence for *in vitro* dedifferentiation but rare proliferation of mouse pancreatic beta-cells. Diabetes. 2007;56:1299-304.
82. D'Alessandro JS, Lu K, Fung BP, Colamn A, Clarke DL. Rapid and efficient *in vitro* generation of pancreatic islet progenitor cells from nonendocrine epithelial cells in the adult human pancreas. Stem Cells Develop. 2007;16:75-89.
83. D'Ippolito G, Diabira S, Howard GA, Menei P, Roos BA, Schiller PC. Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential. J Cell Sci. 2004;117: 2971-81.
84. Tayaramma T, Ma B, Rohde M, Mayer H. Chromatin-remodeling factors allow differentiation of bone marrow cells into insulin-producing cells. Stem Cells. 2006;24:2858-67.
85. Moriscot C, De Fraipont F, Richard M-J, Marchand M, Savatier P, Bosco D, et al. Human bone marrow mesenchymal stem cells can express insulin and key transcription factors of the endocrine pancreas developmental pathway upon genetic and/or microenvironmental manipulation *in vitro*. Stem Cells. 2005;23:594-604.
86. Karnieli O, Izhar-Prato Y, Bulvik S, Efrat S. Generation of insulin-producing cells from human bone marrow mesenchymal stem cells by genetic manipulation. Stem Cells. 2007;25:2837-44.
87. Timper K, Seboek D, Eberhardt M, Linscheid P, Christ-Crain M, Keller U, et al. Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells. Biochem Biophys Res Commun. 2006;341:1135-40.
88. Herrera MB, Bruno S, Buttiglieri S, Tetta C, Gatti S, Deregibus MC, et al. Isolation and characterization of a stem cell population from adult human liver. Stem Cells. 2006;24:2840-50.
89. Sun Y, Chen L, Huo X-g, Hou W-k, Dong J-j, Sun L, et al. Differentiation of bone marrow-derived mesenchymal stem cells from diabetic patients into insulin-producing cells *in vitro*. Chin Med J. 2007;120:771-6.
90. Battula VL, Bareiss PM, Treml S, Conrad S, Albert I, Hojak S, et al. Human placenta and bone marrow derived MSC cultured in serum-free, b-FGF-containing medium express cell surface frizzled-9 and SSEA-4 and give rise to multilineage differentiation. Differentiation. 2007;75:279-91.