

COMMENTARY

Stem cell therapy for diabetes: do we need to make beta cells?

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Abstract

Type 1 diabetes can now be ameliorated by islet transplantation, although this treatment is restricted by the insufficient supply of islet tissue. The need for an essentially limitless supply of a substitute for primary human islets of Langerhans has led to research into the suitability of stem/progenitor cells to generate insulin-producing cells to use in replacement therapies for diabetes. Although there has been much research in this area, an efficient and reproducible protocol for the differentiation of stem cells

into functional insulin-secreting β -cells that are suitable for transplantation has yet to be reported. In this commentary we examine the minimum requirements for replacement β -cells and outline some of the potential sources of these cells. We also argue that the generation of the 'perfect' beta-cell may not necessarily lead to the most suitable tissue for transplantation.

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Introduction

Type 1 (insulin-dependent) diabetes is a chronic disease affecting genetically predisposed individuals, in which insulin-secreting β -cells within pancreatic islets of Langerhans are selectively and irreversibly destroyed by autoimmune assault. For over 80 years the main therapeutic approach to insulin-dependent diabetes has been confined to treating the symptoms by insulin replacement. Recently, significant advances in the transplantation of human primary islets of Langerhans into individuals with Type 1 diabetes has largely removed this insulin dependency (Shapiro *et al.* 2000, Ryan *et al.* 2001). However, the application of this treatment is restricted by the very limited availability of primary human islets from heart-beating donors, and what is now required is an essentially limitless supply of a physiologically competent substitute for primary human islets of Langerhans.

Beta cells from stem cells

Recent advances in stem cell biology raise the possibility of offering personalised therapy to people with Type 1 diabetes by applying cloning strategies to create immunologically autologous embryonic stem (ES) cells from which to generate functional pancreatic β -cells for transplantation therapy. Therapeutic cloning involves taking the nucleus

from one of the patient's somatic cells, inserting it into an enucleated human egg and allowing it to develop into a blastocyst. The inner cell mass of the blastocyst is used to generate pluripotent ES cell lines, which can be expanded *in vitro* to produce the billions of cells required for transplantation therapy. Alternatively, the stem cell populations may be derived by expansion of tissue stem cells from biopsy samples of the patient's pancreas, liver, or bone marrow. Whatever their origin, the stem cells will be differentiated into insulin-producing cells, and these will be formed into islet-like structures for transplantation into the patient to cure their diabetes.

Although seemingly idealistic, many aspects of this scenario are already possible. Human embryos have been cloned using nuclei from somatic cells (Hwang *et al.* 2004), although there are unresolved ethical and legal issues with this process. Pluripotent human ES cell lines have been generated from both cloned and normal blastocysts, and the number of cell lines available to researchers is on the increase (Cowan *et al.* 2004). We have the ability to form islet-like structures from β -cell populations *in vitro* (Hauge-Evans *et al.* 1999), and there are now numerous instances (>250 in over 25 centres worldwide) of islet transplantation in people with Type 1 diabetes. However, the pivotal, and as yet unresolved, stage in this novel therapeutic process is the efficient and reproducible differentiation of stem cells into functional insulin-secreting β -cells.

Minimum requirements for replacement β -cells

Any substitute for primary islets of Langerhans will require some minimum essential properties if it is to be of use in the transplantation therapy of diabetes.

First, vast numbers of replacement β -cells will be required to make any significant therapeutic impact. Thus, current transplantation protocols use up to 1×10^6 primary human islets per recipient, equivalent to approximately $2\text{--}4 \times 10^9$ β -cells. If we multiply this by the number of potential recipients with Type 1 diabetes (up to $\sim 10^5$ in the UK; $\sim 10^6$ in the USA), the scale of the problem becomes apparent. Although a recent report identified pre-existing β -cells as the source of new β -cells in normal growth and development (Dor *et al.* 2004), mature β -cells have a very low proliferative capacity (Swenne 1992). As a result, the large numbers of cells required will have to be derived from a proliferative precursor population that can be expanded considerably *in vitro* before differentiation into the mature β -cell phenotype. The ability of stem cells of adult or embryonic origin to replicate and to differentiate into a range of tissue types makes them attractive candidates for producing replacement β -cells.

Secondly, the replacement cells must have the ability to synthesise, store and release insulin when it is required, primarily in response to changes in the ambient glycaemia. Pancreatic β -cells have evolved intricate mechanisms which allow them to monitor and respond rapidly to changes in circulating nutrients, and these mechanisms are now reasonably well understood (reviewed by Jones & Persaud 1998). Given the complexity of the β -cell glucose-induced stimulus-response coupling mechanism (Fig. 1), it is perhaps not surprising that attempts to engineer some of these response elements into substitute β -cells have so far failed to produce cells with normal secretory phenotypes (reviewed by Persaud 1999).

Thirdly, the proliferative capacity of the replacement cells must be tightly controlled to avoid the development of hyperinsulinaemic hypoglycaemia as the β -cell mass expands *in vivo*. This is not a problem when transplanting non-proliferative primary β -cells, but it has the potential to cause profound problems when using cells derived from proliferative precursors unless precautions are taken to exclude proliferative cells from the transplant material. In our opinion, this precludes the clinical use of the many transformed, proliferative insulin-secreting cell lines that have been reported (see Persaud 1999), and these will not be considered further here.

Finally, the transplanted cells must avoid destruction by the recipient's immune system. The immunology of transplant rejection is a complex area and various strategies are being adopted to avoid the problems of immune responses without resorting to global and life-long immunosuppression. There are, however, specific problems when considering β -cell transplantation into patients

with Type 1 diabetes since their immune systems are programmed to destroy primary β -cells, and will presumably target even the immunologically homologous β -cell replacements that would be derived by therapeutic cloning of embryonic stem cells. One way of circumventing this problem may be to generate insulin-secreting cells that possess the functional phenotype of β -cells but which are developmentally and immunologically distinct from primary β -cells and so may evade the immune assault without immunosuppression.

Potential sources of stem cells

A variety of tissues harbour progenitor or stem cells (summarised in Table 1), and if it were possible to isolate and expand these cells *in vitro* and then differentiate them to adopt a β -cell phenotype, they would be a potential source of substitute tissue for transplantation. The pancreas is an obvious source tissue and a number of studies have suggested the existence of stem cells within the pancreas that can be induced to adopt some elements of a β -cell phenotype (Bonner-Weir *et al.* 2000, Hunziker & Stein 2000, Ramiya *et al.* 2000, Habener 2001, Zulewski *et al.* 2001, Abraham *et al.* 2002, Seaberg *et al.* 2004). Progenitor cells from tissues other than the pancreas have also received considerable attention. Thus, liver and pancreas have a common embryonic origin, share many phenotype-maintaining transcription factors and both are equipped to respond to circulating glucose concentrations. Furthermore, there is evidence demonstrating trans-differentiation of cells from the liver towards a β -cell phenotype (Ferber *et al.* 2000, Susuki *et al.* 2002, Yang *et al.* 2002, Horb *et al.* 2003, Kojima *et al.* 2003, Tuch *et al.* 2003). Similarly, it has been reported that stem cells derived from bone marrow can be differentiated *in vitro* (Jahr & Bretzel 2003) and *in vivo* (Ianus *et al.* 2003) into insulin-expressing cells, although these progenitor cells are unlikely to be the highly proliferative haematopoietic stem cells (Lee & Stoffel 2003). However, to date there is no convincing evidence that insulin-producing cells derived from pancreatic stem cells or liver progenitors can be expanded *in vitro* to clinically useful numbers.

Neural progenitor cells, isolated from regions within the brain, can be significantly expanded *in vitro* (Minger *et al.* 1996) and have the capacity to differentiate from their ectodermal origins into cells from all three germ layers (Clarke *et al.* 2002, Sang *et al.* 2002). This is of particular interest since all of the elements of the β -cell glucose-sensing mechanism (Fig. 1) are also expressed in populations of neurons (Yang *et al.* 1999), as are many of the recognised transcription factors that are used to map the development of the β -cell (Wilson *et al.* 2003). We have recently demonstrated that rat neural stem cells can be expanded *in vitro*, and can be induced to express the

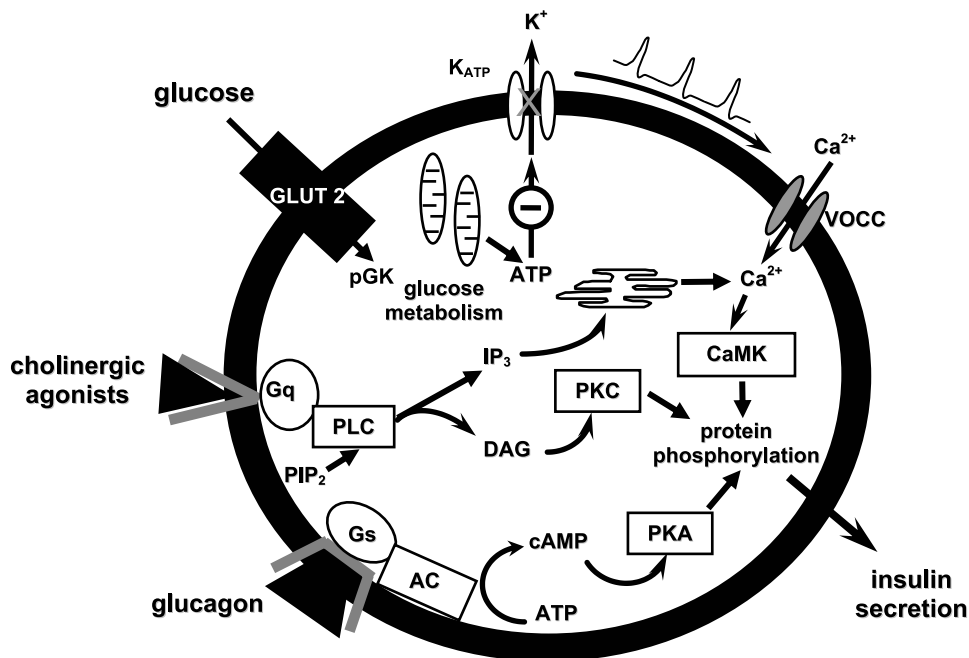


Figure 1 Stimulus-response coupling in a primary pancreatic β -cell. The figure shows a schematic representation of how a β -cell recognises changes in extracellular glucose and translates that recognition into an insulin secretory response. When extracellular glucose increases, glucose is transported rapidly into the β -cell on the high capacity GLUT2 transporter such that cytosolic glucose mirrors plasma glucose. Inside the β -cell, glucose is rapidly phosphorylated by the pancreatic form of glucokinase (pGK) and the subsequent increase in ATP production by mitochondrial oxidative metabolism causes the closure of plasma membrane ATP-sensitive K^+ -channels (K_{ATP}), which are composed of Kir6-2 and SUR1 subunits. The reduced K^+ efflux leads to the depolarisation of the β -cell which, in turn, causes the opening of voltage-operated Ca^{2+} channels (VOCC) in the plasma membrane. Extracellular Ca^{2+} ($[Ca^{2+}]_e$) enters through the VOCC and the consequent elevations in intracellular Ca^{2+} ($[Ca^{2+}]_i$) initiate an insulin secretory response by activating a number of Ca^{2+} -sensitive processes, including protein kinases such as CaMK (calcium/calmodulin-dependent protein kinase) and protein kinase C (PKC), phospholipases and exocytotic elements. The figure also shows how insulin secretion from the β -cell can be modulated by non-nutrient secretagogues such as glucagon (and related peptides) and the cholinergic agonist, acetylcholine. Glucagon binds to a G-protein-associated cell surface receptor coupled to adenylate cyclase (AC), resulting in an increase in cAMP and an activation of protein kinase A (PKA). Acetylcholine binds to a G-protein-associated cell surface receptor coupled to phospholipase C (PLC) which liberates inositol trisphosphate (IP_3) and diacylglycerol (DAG) from membrane phospholipids. IP_3 releases Ca^{2+} from the endoplasmic reticulum and this increase in $[Ca^{2+}]_i$ activates CaMK. DAG, either alone or together with Ca^{2+} , activates PKC. The activation of these protein kinases leads to protein phosphorylation, the stimulation of exocytotic events and ultimately insulin secretion.

insulin gene and respond metabolically to nutrients and sulphonylureas (Burns *et al.* 2003). The ability of neural cells to express the insulin gene is consistent with reports of transient expression of preproinsulin in the fetal brain during development (Devaskar *et al.* 1993*a,b*).

An alternative source of highly proliferative, pluripotent cells which has received much more attention is ES cells. Derived from the inner cell mass of the blastocyst, these cells have the capacity to differentiate into all three embryonic germ layers *in vitro*. A large number of reports have now demonstrated that ES cells can differentiate into cells with an insulin-expressing phenotype, either by genetic manipulation or by permitting spontaneous

differentiation followed by culture under selective conditions (Soria *et al.* 2000, Assady *et al.* 2001, Lumelsky *et al.* 2001, Hori *et al.* 2002, Shiroy *et al.* 2002, Blyszczuk *et al.* 2003, Moritoh *et al.* 2003, Miyazaki *et al.* 2004, Segev *et al.* 2004, Sipione *et al.* 2004). In common with studies using tissue stem cells, the cellular identity of these insulin-expressing cells is uncertain and with the lack of specific, easily identifiable markers of a mature β -cell, the possibility remains that these cells are not fully mature β -cells but instead are a phenotypically similar population of cells, perhaps of neuroectodermal (Lumelsky *et al.* 2001, Burns *et al.* 2003) or extra-embryonic (Houard *et al.* 2003) origin.

Table 1 Type of stem cell available, their pros and cons together with the latest developments and other references are shown

	Tissue progenitor				Embryonic	
	Pancreatic	Liver	Blood	Neural	Mouse	Human
Pros	Committed to pancreatic fate; autograft?	Embryological origin; biochemically similar to β -cell; autograft	Pluripotent?; readily available; expandable; autograft	Pluripotent?; readily available; expandable; phenotypically similar to β -cell	Pluripotent; readily expandable; ease of use	Pluripotent; expandable clinical potential
Cons	Difficult to isolate and expand	Difficult to isolate and expand	Limited data; irreproducible?	Limited data; limited availability of early human foetal tissue	No clinical potential	Difficult to use; legal and ethical constraints
Latest developments	Adult β -cells form by self-duplication not from stem cells (Dor <i>et al.</i> 2004)	Pancreatic factors differentiate liver to pancreas (Kojima <i>et al.</i> 2003)	Mouse bone marrow cells form β -cells <i>in vitro</i> (Tang <i>et al.</i> 2004)	Rodent neural stem cells can be expanded <i>in vitro</i> and induced to express the insulin gene (Burns <i>et al.</i> 2003)	Selection of β -cells by Nlx6.1 expression (León-Quinto <i>et al.</i> 2004)	Human ES cells differentiate into β -cells (Segev <i>et al.</i> 2004)
Other references	Bonner-Weir <i>et al.</i> (2000), Ramiya <i>et al.</i> (2000), Zulewski <i>et al.</i> (2001), Hunziker and Stein (2000)	Ferber <i>et al.</i> (2000), Yang <i>et al.</i> (2002), Kojima <i>et al.</i> (2003), Zalzman <i>et al.</i> (2003)	Lee and Stoffel (2003), Janus <i>et al.</i> (2003), Jahr and Bretzel (2003), Tang <i>et al.</i> (2004)	Burns <i>et al.</i> (2003)	Soria <i>et al.</i> (2000), Lumelsky <i>et al.</i> (2001), Hori <i>et al.</i> (2002), Shiroy <i>et al.</i> (2002), Rajagopal <i>et al.</i> (2003), Blyszczuk <i>et al.</i> (2004), Sipione <i>et al.</i> (2004), León-Quinto <i>et al.</i> (2004)	Assady <i>et al.</i> (2001), Segev <i>et al.</i> (2004)

Stem cell therapy for diabetes

Despite considerable efforts over the past 4 years, we still lack the means for reproducibly and efficiently differentiating stem cell populations into functional β -cells. There are probably many reasons why this has so far proved an elusive goal but, in our view, there are two main issues to be resolved.

(i) Which stem cells?

Perhaps the most important issue is the choice of the appropriate starting material. Research efforts are currently divided between embryonic and tissue stem cells as potential therapeutic progenitor cells. The proliferative capacity of ES cells is attractive, but their pluripotency may be a disadvantage. Differentiation of pluripotent ES cells generally produces a mixture of many different cell types and, in the absence of reliable selection procedures, this process cannot yet produce the homologous populations of fully differentiated β -cells required for transplantation therapy. Tissue stem cells are further down the developmental pathway than ES cells, and so may be lineage-restricted to some extent, which may make it easier to drive them down particular developmental lineages. However, this experimental advantage is currently outweighed by the limited replicative potential of tissue stem cell populations *in vitro*. There may also be more subtle differences in the developmental potential of different stem cell populations. It is becoming evident that ES cell lines, both mouse and human, are not identical in their phenotype, in their culture requirements *in vitro*, or in their propensity to differentiate down particular lineages. This implies that some cell lines may be more appropriate than others from which to generate β -cells. If so, we may need to apply differentiation protocols to numerous ES cell lines to determine which line, if any, is the best starting material. The generation of large numbers of different human ES cell lines, and the development of centralised 'stem cell banks' to characterise, store and distribute the cells should facilitate this process.

(ii) Do we need to make β -cells?

The second question is whether we need to recapitulate *in vitro* the precise developmental pathway that leads to the differentiation of β -cells *in vivo*. Many current studies, particularly those using ES cells, try to map their experimental protocols on to the known developmental pathways of pancreatic endocrine cells. However, the complex sequence of developmental events directing duodenal endoderm towards an insulin-expressing β -cell phenotype *in vivo* are the result of millions of years of evolutionary selection, driven by environmental pressures rather than by conscious design. There is some evidence that it may be possible to employ conscious design to arrive at the same

end-point by a less circuitous route. Thus, it has been suggested that the pathways of β -cell differentiation *in vitro* may differ significantly from those *in vivo* (Houard *et al.* 2003) and mouse ES cells are reported to differentiate into endocrine cells without PDX-1 (pancreatic duodenal homeobox-1) expression (Moritoh *et al.* 2003), although this is essential *in vivo* (Scharfmann 2000). It is also possible that current *in vitro* differentiation protocols do not generate β -cells, but, instead, cells that have some phenotypic and functional similarity to authentic β -cells. For example, it has been suggested that a neural fate is a default pathway for differentiation of ES cells (Tropepe *et al.* 2001), and the application of culture conditions selective for nestin-positive precursors (Lumelsky *et al.* 2001) leads to insulin expression in cells with a neuronal phenotype (Sipione *et al.* 2004). These observations are consistent with our demonstration that neural stem cells can be induced to express mRNAs encoding insulin and elements associated with β -cell function, since these ectodermally derived cells are unlikely to be authentic β -cells. An alternative developmental origin for insulin-expressing cells could be the visceral endoderm of the yolk sac (Rau *et al.* 1989, McGrath & Palis 1997). Visceral endoderm develops initially in a similar manner to embryonic endoderm, and primitive endodermal cells are localised to the periphery of EBs (embryoid bodies) during differentiation of mouse ES cells (Murray & Edgar 2001). It has, therefore, been suggested that the insulin-expressing cells generated during EB formation are from visceral endoderm, since they are located almost exclusively in the outer layer of the EB (Houard *et al.* 2003). In the absence of specific and highly expressed markers for authentic β -cells it is difficult to determine unambiguously the origin of insulin-expressing cells generated *in vitro* from pluripotent progenitor populations. In any event, in our opinion, the precise developmental identity of the cells used for transplantation therapy may not be important so long as they offer an expandable population of cells that fulfils the functional criteria of replacement β -cells.

Conclusion

Type 1 diabetes is now curable by transplantation therapy, and stem cells offer a potential starting material from which to generate the large numbers of cells required. To date, most efforts have attempted to drive pluripotent stem cells down an endodermal, pancreatic, endocrine lineage but we suggest that it may not matter whether the replacement cells are developmentally authentic pancreatic β -cells as long as their functional phenotype is sufficient to produce physiological patterns of insulin secretion. Indeed, there is every expectation that the immune system in the transplant recipient with Type 1 diabetes is primed selectively to destroy even immunologically autologous pancreatic β -cells. It may, therefore,

be a more sensible long-term strategy to focus efforts on generating populations of cells that possess the essential attributes of β -cells but which will not be recognised as β -cells by the recipient's immune system.

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