



# Cellular sources of new pancreatic $\beta$ cells and therapeutic implications for regenerative medicine

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**Replacing missing insulin-producing  $\beta$  cells to treat diabetes is a major challenge for regenerative medicine. A better understanding of  $\beta$ -cell embryogenesis and regeneration in adult life is needed to devise means to derive these specialized cells in sufficiently large numbers from stem or precursor cells. It is also critical to ensure that any surrogate or regenerated  $\beta$  cells have perfectly regulated insulin production, which is essential for physiological glucose homeostasis.**

In August 2004, the British government awarded a licence to the Newcastle Fertility Centre for therapeutic cloning; generation of insulin-producing cells to treat diabetes was mentioned as a major goal. Although the authorization itself will be challenged on ethical grounds by some, the choice of disease will surely not. Diabetes is invariably mentioned as one of the most attractive targets for stem-cell therapy or regenerative medicine and there are sound reasons for this (even if the research community and media would be well-advised not to raise false hopes at this early stage<sup>1</sup>). This perspective will summarize these reasons and will examine whether the field is really in a strong position to deliver on its promises.

## Setting the scene: Why diabetes is so serious and how useful cell replacement therapy could be

Diabetes in all its forms currently afflicts at least 200 million people in the world and this number is expected to double by the year 2025. It is a chronic disease with severe secondary complications driven by poor glycaemic control and for which there is as yet no cure. Type 1 diabetes — which is responsible for less than 10% of the total population of individuals with diabetes, and may become manifest at any age, even if there does seem to be a peak in early adolescence — results from autoimmune destruction of insulin-secreting

$\beta$  cells in the pancreatic islets of Langerhans. Survival depends on multiple daily insulin injections.  $\beta$ -cell replacement therapy could, in principle, provide a cure for Type 1 diabetes even if there are formidable obstacles as discussed below. Islet transplantation has been impressive in rendering some patients insulin-independent for a number of years, but this requires possibly toxic immunosuppression and there will never be enough donor islets to satisfy demand. Type 2 diabetes — which is often but not always associated with obesity and is now becoming manifest in increasingly younger individuals — is caused by the combination of insulin resistance and inadequate insulin secretion, and is treated initially by oral hypoglycaemic agents typically with adjunct insulin therapy in the later stages of the disease. Indeed, it is now well accepted that  $\beta$ -cell mass is decreased by about 50% in Type 2 diabetes. Replacing these missing cells could be a means to treat the insulin delivery malfunction of this form of the disease<sup>2</sup>.

Let us consider possible strategies for generating large numbers of  $\beta$  cells *in vitro* for cell-replacement therapy and prospects for regeneration of the endocrine pancreas in individuals with diabetes.

## Derivation of insulin-producing cells from embryonic stem cells *in vitro*

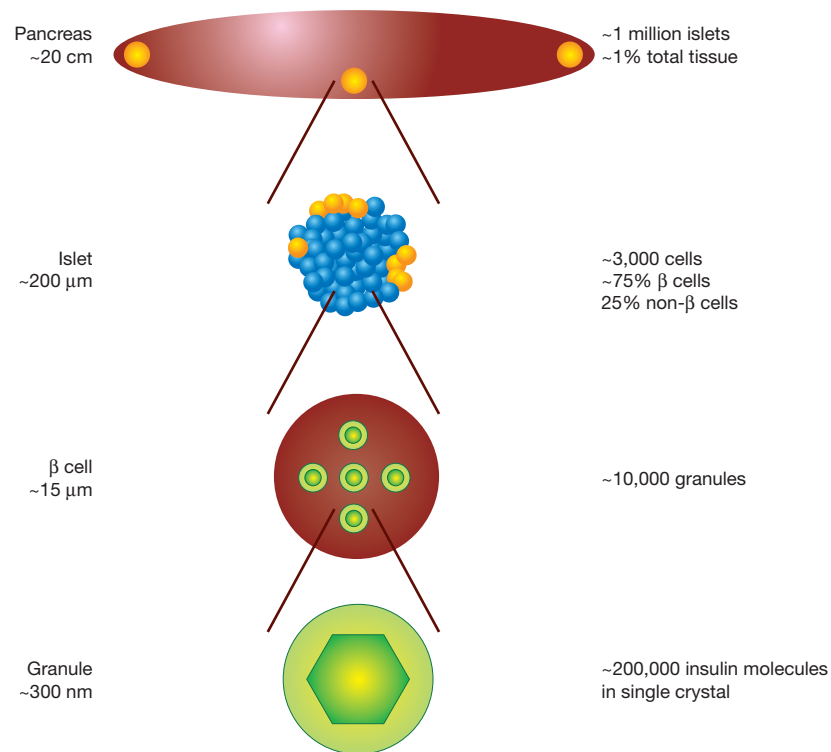
Many of the major *trans*-acting factors implicated in  $\beta$ -cell embryogenesis have been identified, and the timing of their activation or repression charted<sup>3,4</sup>. There may not yet be a complete picture, but it's getting closer. This information has been useful for initial attempts to derive insulin-secreting cells from mouse

and human embryonic stem cells (ESCs). The diverse pathways and regimens used for this purpose and their respective promises or problems have been reviewed recently<sup>5,6</sup>. There are still major obstacles to be overcome before insulin-producing cells derived in this way can be used therapeutically: typically, there is a mixed population of cells with only a minority producing insulin; insulin content per cell is lower than in adult human  $\beta$  cells; regulation of secretion is not fully physiological; differentiated ESCs survive poorly; and most studies have depended on mouse ESCs. However, these studies do provide first proof of principle and initial protocols may function as a template for refinement and increased efficacy<sup>7</sup>. Improved understanding of islet morphogenesis and new techniques for *in vitro* expansion and differentiation of human ESCs, combined with the availability of new and better-defined ESC cell lines and the advent of therapeutic cloning, may introduce an entirely new dynamic to the field. Stay tuned!

## On the origin of new $\beta$ cells in adult man and implications for regenerative medicine

It is unfortunate that very much less is known about the potential for generating new  $\beta$  cells in adult humans and the origin of any such newly formed cells than about islet embryogenesis. Yet this will be an essential key to any strategy for *in situ* therapeutic  $\beta$ -cell regeneration in diabetes. Despite pioneering studies in the 1960s (reviewed in ref. 8) suggesting  $\beta$ -cell regeneration in a variety of situations in the rat at all stages of life, the adult endocrine pancreas was mistakenly long considered quiescent.

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**Figure 1** Insulin production from organ to molecule. Islets constitute only 1–2% of total pancreatic tissue, and over 1 million islets are dispersed throughout the organ in adult humans. Each islet consists of several thousand cells of which the majority are  $\beta$  cells. Each  $\beta$  cell carries an ample reserve of insulin stored in

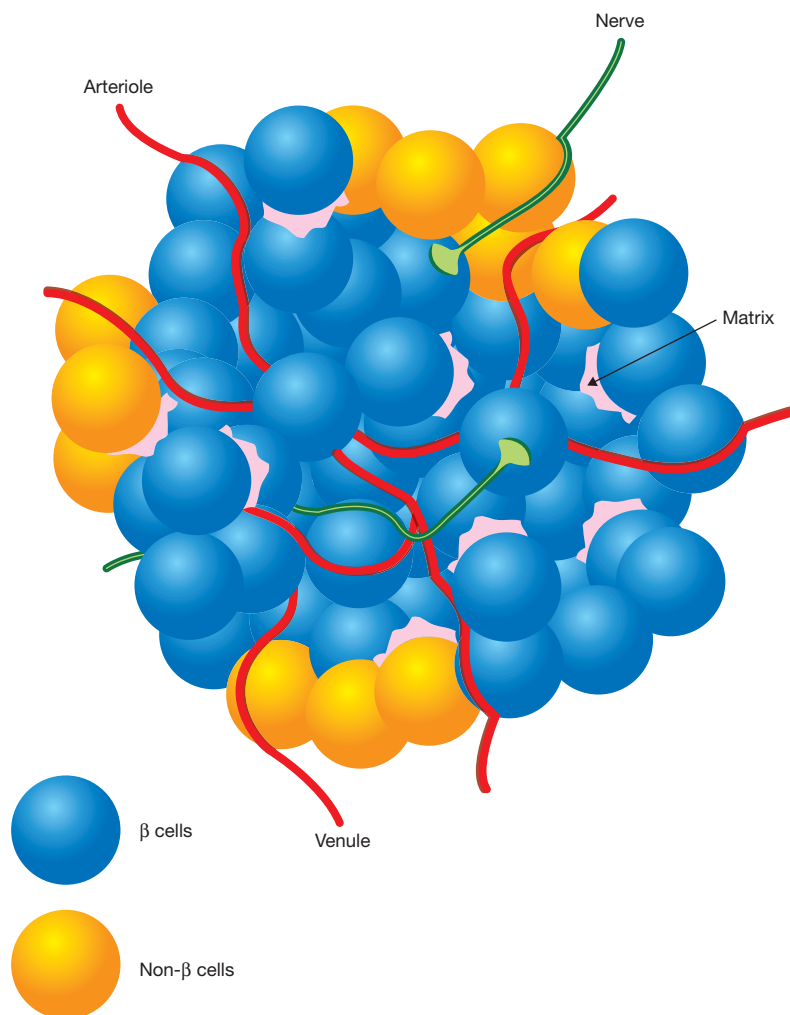
secretory granules that will release their contents by exocytosis in response to an appropriate stimulus including glucose, a major  $\beta$ -cell secretagogue. In most mammalian species, including humans, insulin is stored within granules as a crystal. Estimated numbers were derived in part from ref. 29.

More recent studies have confirmed and extended the earlier work, showing that changing metabolic demand is matched by increased or decreased  $\beta$ -cell mass<sup>9</sup>. In humans,  $\beta$ -cell mass increases in obese individuals but is decreased in weight-matched individuals with diabetes<sup>10</sup>. Cell mass is, of course, the reflection of the dynamic balance between rates of cell generation and death (without considering for the present purposes the possible additional contribution of hypertrophy, or increase in cell size rather than number). Increased  $\beta$ -cell mass could thus reflect decreased death (owing largely, it is believed, to apoptosis) rather than increased generation or perhaps a combination of the two.

If regeneration does occur, it is not entirely clear whether this is by self-replication or *bona fide* neogenesis; that is, expansion and differentiation of precursor cells or *trans*-differentiation. Studies in the rat, and notably following physical or chemical insult — although less

so during pregnancy — suggest that even in adult life islet regeneration may be the result of the combination of replication of existing  $\beta$  cells and neogenesis (derivation of new  $\beta$  cells from a non- $\beta$ -cell precursor), even if the latter may be of lesser importance with increasing age<sup>11</sup>. Formal proof of neogenesis will, however, depend on cell tracing or lineage studies. It is believed by some that in the rat, islet precursor cells reside in the pancreatic ducts, whereas others believe that acinar cells may de-differentiate to a more primitive state followed by re-differentiation into islet cells<sup>12</sup>. Islet stem cells may, of course, reside outside and migrate to the pancreas. Bone marrow cells give rise to insulin-producing cells in the pancreas<sup>13</sup> (and even in other organs in a hyperglycaemic animal<sup>14</sup>) although this idea has been contested<sup>15</sup> and one other study shows that bone marrow cells favour replication of existing  $\beta$  cells<sup>16</sup>. Most recently, the field has been challenged by an elegant genetic

lineage study claiming that in adult mice, even following partial pancreatectomy to trigger regeneration, replication of existing  $\beta$  cells is the overwhelmingly dominant pathway for the formation of new  $\beta$  cells<sup>17</sup>. The authors conclude that their study closes the door on the existence (or at the least the physiological relevance) of  $\beta$ -cell precursors or stem cells in the adult mouse: they state that at a certain moment in postnatal life the number of islets is set and that “... $\beta$ -cell formation from undifferentiated precursors ceases at this time...”. There is some concern in the community that such categorical wording may not be justified by the data, and most specifically given that the study was not designed to identify precursor cells *per se* (and cannot prove their absence, as mentioned by the authors) but rather to provide evidence for or against their participation in  $\beta$ -cell regeneration under quite select circumstances that are known to result in only modest regeneration. Indeed, an



**Figure 2** The three-dimensional architecture of an islet. Islets of Langerhans are composed of insulin-producing  $\beta$  cells and of other endocrine cell types secreting glucagon, somatostatin and pancreatic polypeptide. These various cell types are organized in a specific fashion, typically with the  $\beta$  cells

forming a more or less well defined central core. Islets are richly irrigated by a well developed microvasculature and endocrine cells are in contact with extracellular matrix (presumably deposited, at least in part, by neighbouring endothelial cells) as well as being selectively innervated.

even more recent study demonstrates clonal derivation of multipotent precursor cells of both islet and ductal origin<sup>18</sup>. All major islet endocrine cell types could be derived from these precursors even if those cells producing insulin cannot at this stage be considered *bona fide* and fully differentiated  $\beta$  cells. It remains to be seen how the apparent conflict between these two studies can be resolved. A fundamental difference of obvious importance for regenerative or cell-based therapy of diabetes, respectively, is that the first was performed *in vivo*<sup>17</sup> and the second *in vitro*<sup>18</sup>. Regardless, rats and mice may not be good models for islet growth and/or regeneration in humans. Obviously, prospective studies, let alone those demanding damage to the endocrine pancreas to induce regenerative repair, cannot be performed in humans. Interestingly, close inspection of tissue sections does reveal the presence of isolated insulin-positive cells in associa-

tion with pancreatic ductules not only in the fetus or newborn, but even in adult human pancreas<sup>19</sup>, whereas there is little evidence for  $\beta$ -cell replication in adult humans. The jury is still out in this case.

#### Derivation of $\beta$ cells from adult human pancreatic precursor cells *in vitro*

Human insulin-producing cells can be derived *in vitro* from precursors and notably from pancreatic duct cells<sup>20,21</sup>. Some would of course argue that this is due to proliferation of contaminating  $\beta$  cells<sup>17</sup> even if there was evidence at least in one study of the need to trigger recapitulation of the embryonic  $\beta$ -cell differentiation pathway<sup>22</sup>. The overriding problem at present, as for derivation of  $\beta$  cells from ESCs, is quantitative. To be clinically relevant, any protocol for *in vitro* amplification/generation of  $\beta$  cells from adult human pancreas must result in sufficient numbers of

fully differentiated cells to allow many individuals to be treated with cells derived from a single donor. At present, nobody seems close to achieving this goal using human pancreas for  $\beta$ -cell derivation *in vitro*.

#### The liver as a possible extra-pancreatic source of $\beta$ cells

Studies demonstrating insulin production in the liver have caught the attention of the community. Clearly, the ability to drive extra-pancreatic cells to express insulin and ultimately to assume a true  $\beta$ -cell phenotype could be useful not only in the context of generating large numbers of cells for implantation but also for (ectopic) regeneration of  $\beta$  cells *in vivo*. Insulin expression was observed in a limited number of cells with reversal of diabetes after *in vivo* infection of the liver of hyperglycaemic mice with recombinant adenovirus expressing the pancreatic and duodenal

homeobox gene 1 (*PDX-1*, also known as *IPF-1*)<sup>23</sup> or by expression of this same factor along with telomerase in fetal human progenitor liver cells *in vitro* followed by implantation in hyperglycaemic mice<sup>24</sup>. The latter study seems particularly encouraging for possible future cell-based therapy of diabetes in that human cells with impressive replicative capacity were used. The first study is also captivating in that it suggests adult liver may also provide a source of  $\beta$  cells. As for the other approaches discussed above, these are early days and the dual problems of quantity (number of insulin-producing cells and the amounts of insulin they produce) and quality (cells derived so far are not fully differentiated  $\beta$  cells) remain. It will, however, be interesting to follow developments in this approach, possibly involving expression of additional key regulatory genes to drive the cells further down the  $\beta$ -cell lineage. It will also be interesting to discover the origin of insulin-producing cells in the liver; for example, they may arise from pluripotent liver precursor cells, from the occasional bone marrow-derived stem cell or perhaps as a result of true *trans*-differentiation of a limited number (or subpopulation) of hepatocytes.

### The natural environment of the $\beta$ cell and differentiated function: the great challenge

The combination of morphology and cell and molecular biology has allowed  $\beta$ -cell function to be described in astonishing detail. This neuroendocrine cell is typified by an ample reserve of insulin stored in secretory granules and available for quantal secretion at a moment's notice and in response to the metabolic needs of the individual. Both under- and over-insulinization are, in the extreme, life-threatening, leading to hyper- and hypoglycaemia, respectively. Replacement  $\beta$  cells will have to perform as well as their natural counterparts to be of clinical use as well as adapting to changes in insulin demand with time<sup>25</sup>. These unique features of the  $\beta$  cell are more often than not overlooked, or at the least under-emphasized by non-specialists in their various attempts to create these cells *in vitro* or to regenerate them *in vivo*<sup>26</sup>. The impact of the natural environment of the  $\beta$  cell on its function is a confounding issue adding a further degree of complexity and raising the stakes yet higher.  $\beta$  cells thus reside naturally in the islets of Langerhans, micro-organs consisting of a few thousand endocrine cells, of which some 75% are  $\beta$  cells, the rest secreting glucagon, somatostatin or pancreatic polypeptide (Fig. 1). Islets are richly irrigated and enervated. Their characteristic cellular architecture allows for specific intercellular communication that, in

combination with other outside-in signals, is critical for well regulated insulin secretion (Fig. 2). Disturbing the social life of the  $\beta$  cell will most probably have a profound negative effect on its function. This knowledge is not new<sup>27</sup>. Driving *in situ* regeneration of, or replacement therapy using, isolated  $\beta$  cells or of a collective of  $\beta$  cells deprived of their natural neighbours may not result in a clinically satisfactory solution and may even be unsafe if ever insulin secretion is severely disturbed<sup>25,26</sup>. The greater challenge may then be the need to recreate islets, with their normal endocrine cell architecture, microvasculature (allowing for physiological irrigation and for close contacts between endocrine cells and extracellular matrix deposited by endothelial cells) and enervation, rather than  $\beta$  cells. At the very least, producing a cell that synthesizes insulin is just one part of a much more complex equation that may one day lead to the creation of a true  $\beta$ -cell surrogate of clinical relevance.

### New therapeutic approaches must be safe and offer significant improvement over current therapy

Regardless of the origin of newly generated insulin-secreting cells, there are common deliverables that must be in place for clinical use. The standards have been set quite high<sup>26</sup> and to my knowledge, no system or approach, however promising, fulfils all these requirements at present. If diabetes is indeed devastating, modern therapy has greatly improved the quality of life of any individual with the disease who is fortunate enough to live in a developed country. Treatment of Type 1 diabetes has improved with the availability of increasingly versatile insulin analogues and (most importantly to patients although perhaps seemingly banal to the average cell biologist in good health) sharp needles and home glucose monitoring. Individuals with Type 2 diabetes have greatly improved glycaemic control using contemporary oral hypoglycaemic agents often in combination with insulin injection. However, most patients and their families will tell you that they continue to live in hope of improved therapy, ideally dispensing with injections and providing a life-long cure. Any new therapy must (obviously) be as safe — if not safer — and as good — if not better — than what we have at present. Ideally, it should be cheaper and readily available to the rapidly growing population with diabetes in less developed countries. Finally, it must not depend on immunosuppression while allowing for new  $\beta$ -cells in the hostile (autoimmune) environment of an individual with Type 1 diabetes.

The race is on to provide a plentiful supply of fully functional  $\beta$  cells to individuals with diabetes. The initial rush to publication of the first observations of newly derived or regenerating  $\beta$  cells is over and we are now entering a more challenging and rigorous period of consolidation that must be combined with innovative and multi-disciplinary research in basic cell and developmental biology. The knowledge base is there even if there are some critical gaps to be filled in. In any event, the pathway to success and the origin of any replacement  $\beta$  cell will ultimately be less important than the quality and performance of the product. As in life, it is not about where you come from and who your parents are; it is about who you are and what you do. The  $\beta$  cell will be a tough act to copy: the nature (and beauty) of the beast and of insulin itself requires us to be copy perfect. Some have gone further and suggested we may be able to improve on the natural  $\beta$  cell itself<sup>28</sup> — now there is a truly bold new challenge.

### ACKNOWLEDGEMENTS

P.A.H. is supported by the Juvenile Diabetes Research Foundation, the National Institutes of Health and the Swiss National Science Foundation.

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