

Replenishing a Balanced Mixture of Hormone Producing Cells: A Necessary Component in the Stem Cell Based Therapy for Diabetes?

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Patients with type 1 diabetes produce little or no insulin due to an autoimmune destruction of the β -cell mass. Several decades ago, it has been demonstrated that diabetic rats could restore normoglycemia after transplantation with isogenic islets [1]. This observation resulted in whole-pancreas or islet transplantation to treat patients with type 1 diabetes [2]. Nevertheless, several hurdles still need to be resolved before this strategy can be introduced for routine use. The major problem is the scarcity of transplantable islets. Therefore, several groups are currently trying to generate large numbers of β -cells *in vitro* from stem cells of both embryonic and adult origin for clinical utility [3]. Regarding this aspect, a lot of progress has been made and the company Viacyte optimized a reproducible method to generate implantable material from Cyt49 human embryonic stem cells, which is suitable for industrialization [4].

However, before going to therapy, several questions need to be resolved. Beside important safety issues (risk for the development of teratomas) and issues regarding the need of immunosuppressive drugs, it is also not clear how many β -cells ideally need to be transplanted and which cell type, derived from stem cells is the most suitable for transplantation. Although the answers to those questions remain elusive, we will discuss some aspects regarding those issues that will have to be taken into account.

It is well established that sufficient numbers of islets need to be engrafted to restore normoglycemia in diabetic recipients. However, due to a limited availability of islets for transplantation only few studies have addressed what would happen if too many cells are administered. It has been suggested that despite an increased β -cell mass, normoglycemia can still be achieved by a decrease in the insulin secretion on a per β -cell basis [5]. Alternatively, insulin secretion might remain elevated in normoglycemic animals due to decreased insulin sensitivity, as seen in some animal models with an increased β -cell mass [6]. To formally test what would happen if too many β -cells are engrafted, 1,000 syngeneic islets were engrafted under the kidney capsule of 10 to 12 week old FVB and C57Bl/6 mice [7]. After an initial drop in the glycemia during the first 2-3 days, the transplanted mice restored normoglycemia, despite being hyperinsulinemic. Hyperinsulinemic euglycemic clamp experiments demonstrated that under those conditions normoglycemia is maintained by hepatic insulin resistance. Some degree of plasticity was also observed in the endogenous β -cell mass from the mice transplanted with excess β -cells. While the transplanted β -cell mass did not decrease for up to 2 months after transplantation, the endogenous β -cell mass was significantly decreased. Nevertheless, this decrease was not sufficient to prevent hyperinsulinemia in the transplanted animals. Transgenic mice over expressing the proto-oncogene *Pleomorphic adenoma gene 1 (PLAG1)* in the β -cells have an endogenous increase of the β -cell mass and show a similar phenotype as mice with an ectopic increase of the β -cell mass. They also remain normoglycemic despite being hyperinsulinemic due to hepatic insulin resistance [7]. Those experiments suggest that not only sufficient numbers of β -cells need to be transplanted but that transplantation of too many β -cells might lead to hepatic insulin resistance. Thus a proper dosage of the transplanted β -cell mass might be necessary when designing stem cell-based islet replacement therapies.

It is also not clear which cell type derived from hESC ideally need to be transplanted. The differentiation of hESC towards fully matured functional β -cells still remains very challenging. So far, nobody reported an *in vitro* differentiation protocol capable of generating sufficient amounts of exclusively insulin-producing cells meeting all essential criteria of functional β -cells as reviewed by Naujok et al. [8]. Nevertheless, it has been demonstrated that upon transplantation of pancreatic progenitors derived from hESC, the cells can mature further *in vivo* into glucose-responsive, insulin-secreting cells, restoring normoglycemia in diabetic animals. However, this *in vivo* maturation process takes several weeks. Refinement of current protocols to enable the generation of functionally competent β -cells entirely *in vitro* might be an alternative approach. Most likely, upon differentiation of stem cells into pancreatic β -cells, mixtures of β -, α -, and δ -cells will be generated and grafted. It might be important to take the balance of the different endocrine cells present in the transplant into account. Indeed, transgenic mouse models over expressing *PLAG1* in different compartments of the pancreas differentially affect the hormone balance and have a different impact on the glucose homeostasis [9]. The Cre-inducible *PLAG1* transgenic mouse model was intercrossed with *Pdx1-Cre*, *Ngn3-Cre* and *Rip-Cre* transgenic to target *PLAG1* expression to different compartments of the pancreas (the whole pancreas, the endocrine cells and the β -cells, respectively). Those intercrossings resulted in P1-Pdx1Cre, P1-Ngn3Cre and P1-RIPCre mice, respectively. All mice developed islet hyperplasia, but with a different balance of the endocrine hormone-producing cells. In young P1-Pdx1Cre mice (less than 15 weeks), there was a balanced increase in the pancreatic content of insulin and somatostatin, which was associated with normoglycemia. In older P1-Pdx1Cre mice the pancreatic content of somatostatin far exceeded that of insulin, leading to severe hypoglycemia. In contrast in P1-Ngn3Cre mice and in P1-RIPCre mice, the pancreatic content of insulin exceeded that of somatostatin and those mice remained normoglycemic. The hypoglycemia in old P1-Pdx1Cre mice, but not in young P1-Pdx1Cre, P1-Ngn3-Cre and P1-RIPCre mice can possibly be explained that somatostatin can decrease insulin resistance [10]. By the huge increase in somatostatin which might prevent insulin resistance to occur. Indeed, it has been described alternatively, other hormones might contribute to the hypoglycemia in older P1-Pdx1Cre mice as well. Besides the *PLAG1* transgenic mouse models, other mouse models such as those with mono- or biallelic loss of *Arx* and/or *Pax4* also result in specific hormonal imbalances leading to normoglycemia,

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hypoglycemia or even hyperglycemia depending on the levels of the different hormone-producing cells [11]. The data obtained from those different mouse models suggest that it is important to take the balance of the different endocrine cells into account to prevent hypoglycemia in diabetic patients treated by transplantation of stem cells differentiated to the pancreatic lineage.

In conclusion, the current advances in regenerative medicine provide hope for the development of a real cure for diabetic patients. It is clear that sufficient amounts of cells need to be transplanted to restore normoglycemia in diabetic patients, but on the other hand transplantation of too many cells might result in hepatic insulin resistance. Thus the number of transplanted cells should be appropriately determined. Furthermore, the right balance of different endocrine hormone-producing cells should be preserved in order to prevent hypoglycemia in the transplanted patients.

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