

# Prospects for Treatment of Type 2 Diabetes by Expansion of the $\beta$ -Cell Mass

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**T**ype 2 diabetes is a polygenic disease exacerbated by environmental factors, such as obesity and a sedentary life style. The earliest detected metabolic abnormality is insulin resistance. However, insulin resistance alone does not cause diabetes if it is compensated by increased insulin production. The pancreatic islet  $\beta$ -cells initially respond to insulin resistance by increasing their capacity for insulin biosynthesis and secretion. When the existing  $\beta$ -cell mass cannot cope with the demand for insulin, an expansion of the  $\beta$ -cell mass through replication or neogenesis occurs. Type 2 diabetes is caused by the inability of  $\beta$ -cells to compensate sufficiently for insulin resistance. It is likely that this results from impairments in both expansion of the  $\beta$ -cell mass and glucose-induced insulin secretion from  $\beta$ -cells. Current therapy of type 2 diabetes includes a modification of life style, such as diet and exercise, and the use of a variety of pharmacological agents that target increased insulin secretion, decreased hepatic glucose production, and increased sensitivity to insulin. Despite these approaches, a significant number of patients with type 2 diabetes require treatment with exogenous insulin. In principle, expansion of the  $\beta$ -cell mass by stimulation of  $\beta$ -cell neogenesis in the patient, as well as by  $\beta$ -cell transplantation, may facilitate the treatment of type 2 diabetes.

The emerging understanding of embryonic  $\beta$ -cell development and  $\beta$ -cell replenishment in the adult from pancreatic stem cells found in the pancreatic ducts holds the promise of developing future approaches for stimulating  $\beta$ -cell regeneration. These approaches may involve the local delivery of growth factors or other extracellular mitogenic agents, as well as the transfer of genes into duct or islet cells that can modulate their replication and differentiation. However, in evaluating these prospects, the causes of the proliferative and the functional defects of  $\beta$ -cells in type 2 diabetes should be considered. These defects may be genetic and thereby serve as primary contributing factors to the development of hyper-

glycemia and/or acquired as a result of obesity (lipotoxicity) and chronic hyperglycemia (glucotoxicity). If the defects in  $\beta$ -cell neogenesis are primarily genetic, then it is possible that type 2 diabetic patients are not capable of normal  $\beta$ -cell neogenesis. The ability to induce efficient de novo formation of  $\beta$ -cells in these patients may depend on the prospect of understanding these defects and finding ways to overcome them. Similarly, if the functional defects in  $\beta$ -cells are primarily genetic, then the  $\beta$ -cell mass expanded from endogenous sources may be unable to restore normal insulin secretion. Both of these problems can be circumvented by providing  $\beta$ -cells from an exogenous source through transplantation, as in type 1 diabetes. However, analogous to type 1 diabetes, in which transplanted  $\beta$ -cells are likely to be exposed to recurring autoimmunity, in type 2 diabetic patients, the transplanted  $\beta$ -cells may be exposed to conditions that could cause a gradual deterioration in  $\beta$ -cell function. These factors in the diabetic milieu of the patient, which could be toxic or inhibitory to  $\beta$ -cells, are likely to be equally challenging to  $\beta$ -cells generated in the patient by neogenesis. Such conditions could include systemic hyperglycemia, systemic high levels of adipocyte products, such as leptin and free fatty acids, and local deposits of islet amyloid polypeptide, which is co-released by  $\beta$ -cells during insulin secretion and accumulates around cells chronically stimulated to release insulin.

The immunological barriers to  $\beta$ -cell transplantation in type 2 diabetes are far less formidable compared with type 1 diabetes, in which recurring autoimmunity is a central problem. With the development of cell encapsulation techniques, allogeneic cell transplantation in immunobarrier membranes may be accomplished without immunosuppression. However, as with type 1 diabetes,  $\beta$ -cell transplantation on a large scale will require the development of new and abundant sources of  $\beta$ -cells, because transplantation cannot rely on human islet donors to provide cells for >5% of the population afflicted by diabetes. These sources could include human  $\beta$ -cell lines developed by reversible immortalization of human islets in culture, as well as  $\beta$ -cells derived from various precursor cells expanded in culture and induced to differentiate into mature  $\beta$ -cells.

The induction of  $\beta$ -cell replication through reversible immortalization has been shown in mouse  $\beta$ -cell lines as a reliable approach for  $\beta$ -cell expansion without loss of differentiated  $\beta$ -cell function (1,2). The ability to induce growth arrest by a tight regulatory system allows both control of cell number and restoration of cell differentiation, as judged by insulin production and regulated secretion. Induction of  $\beta$ -cell replication often results in partial de-differentiation. Most highly specialized cells must withdraw from the cell cycle to manifest fully their differentiated phenotype. Con-

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ES, embryonic stem.

versely, when induced to resume replication, the cells may turn off genes related to differentiation to allow expression of the genetic program required for cell replication. We used differential hybridization to cDNA arrays to reveal the full range of changes in gene expression between proliferating and quiescent  $\beta$ -cells in reversibly immortalized mouse  $\beta$ -cells (3). The results showed a complex pattern of gene activation and shut-off, which supports the view that proliferating and quiescent  $\beta$ -cells express a significantly different array of genes. In addition to the changes detected at the mRNA level, it is likely that numerous changes occur at the translational and posttranslational levels, which cannot be detected by this analysis. The ability to induce conditional growth arrest in  $\beta$ -cell lines may allow the gene expression pattern to revert to that of a normal differentiated  $\beta$ -cell.

The alternative route to induction of replication in differentiated  $\beta$ -cells is the induction of differentiation into  $\beta$ -cells of various progenitor cells that maintain a substantial proliferative capacity. Recent reports on the generation of human embryonic stem (ES) cell lines (4) have raised hopes for the generation in tissue culture of various differentiated human cell types for transplantation, including insulin-secreting cells (5). The development of nuclear transfer from somatic cells into oocytes in large mammals (6) opens the possibility of generating self-ES cell lines from adult patients, thus avoiding immunological problems of transplant rejection. ES cells can easily be propagated in tissue culture in large numbers and can be modified by gene transfer to correct their genetic defects and optimize their properties. Another attractive source of progenitor cells for generation of  $\beta$ -cell lines is represented by the committed pancreatic stem cells found in pancreatic ducts (7,8). Although their proliferative capacity may be reduced compared with that of ES cells, they may prove to be easier to turn into mature  $\beta$ -cells, given their partial differen-

tiation. The ability to induce and maintain a full-fledged  $\beta$ -cell phenotype, as characterized by the amounts of insulin produced and stored in the cells and its secretion in response to physiological stimuli, remains a major obstacle in using progenitor cells. We need to learn much more about the extracellular signals and regulator genes involved in generating the  $\beta$ -cell phenotype to be able to induce it fully in early progenitor cells. In addition, the use of self-progenitor cells will be limited if the functional impairments of  $\beta$ -cells in type 2 diabetes involve genetic components, unless the defective genes are identified and can be corrected by gene transfer into the progenitor cells during their propagation in tissue culture.

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