Gastrointestinal Stem Cells

I. Pancreatic stem cells

Bernat Soria,1,3 Francisco J. Bedoya,2,3 and Franz Martin1

1Institute of Bioengineering, University Miguel Hernandez, Alicante; 2Department of Environmental Sciences and Laboratorio Andaluar de Terapia Celular y Diabetes, University Pablo Olavide and Junta de Andalucia, Seville; and 3Andalusian Center for Molecular Biology and Regenerative Medicine, Seville, Spain

Soria, Bernat, Francisco J. Bedoya, and Franz Martin. Gastrointestinal Stem Cells. I. Pancreatic stem cells. Am J Physiol Gastrointest Liver Physiol 289: G177–G180, 2005; doi:10.1152/ajpgi.00116.2005.—The transplantation of islets isolated from donor pancreas has renewed the interest in cell therapy for the treatment of diabetes. In addition, the capacity that stem cells have to differentiate into a wide variety of cell types makes their use ideal to generate β-cells for transplantation therapies. Several studies have reported the generation of insulin-secreting cells from embryonic and adult stem cells that normalized blood glucose values when transplanted into diabetic animal models. Finally, although much work remains to be done, there is sufficient evidence to warrant continued efforts on stem cell research to cure diabetes.

Diabetes is a heterogeneous disorder affecting more that 200 million people in the world, and this number is expected to double by the year 2025. Nowadays, diabetes is mostly treated by insulin therapy or oral hypoglycemic agents. However, these treatments cannot stop the severe complications associated with diabetes. These complications include retinopathy, nephropathy, neuropathy, arteriosclerosis, and heart disease along with a considerable reduction of the quality and length of life. A large multicentric study, the Diabetes Control and Complications Trial (13), has demonstrated that achieving normal glucose homeostasis would be a major advancement in the treatment of diabetes. This can best be done by replacing the missing β-cells destroyed in the pancreatic islets of Langhans. Therefore, β-cell replacement could, in theory, provide a solution for diabetes. The transplantation of cells, tissues, and organs to restore adequate physiological responses and/or anatomical structures is an old idea in medicine that became a reality with the discovery of modern immunosuppressive techniques. Despite the success in surgical procedures and the discovery of new immunosuppressors, this approach will always be limited by the scarcity of donors. This is the case of islet transplantsations from human donor pancreas, which have been very successful since 2000 (22). Thus stem cells, with their high potential for proliferation and differentiation, are the hope in future regenerative medicine. In fact, some diseases such as diabetes, in which the damage is localized to a specific cell type, seem to be good candidates for cell therapy.

In this review, we discuss the different strategies used to generate β-cells from stem cells (Fig. 1). Embryonic and adult stem cell approaches are considered, because we think that both are realistic alternative sources of β-cells.

FROM EMBRYONIC STEM CELLS TO INSULIN-PRODUCING CELLS

The establishment in culture of pluripotent cell lines isolated from mouse blastocysts was reported early in the 1980s. These cell lines showed the capability to differentiate in vitro and in vivo (6). Since then, substantial improvements have been achieved in the maintenance of these cells in an undifferentiated state and in the control of their differentiation toward a variety of cell types. The generation of cultures of embryonic stem (ES) cells remaining in a pluripotent state requires the culture of cells from the inner cell mass of preimplantational blastocysts in a culture medium supplemented with fetal calf serum, feeder cells (which provide factors for their in vitro growth), and the leukemia inhibitory factor (LIF). Under such conditions, ES cells aggregate into colonies consisting of a mixed population of undifferentiated cells and spontaneously differentiating cells. After several days to a week, colonies are removed, trypsinized, and plated into new culture dishes. The self-renewal of ES cells requires the binding of LIF to a two-part receptor consisting of an LIF receptor and a gp130 receptor. Receptor activation triggers the JAK/STAT3 proliferative pathway. The activation of ERK by gp130 and other cell-surface receptors counteracts the effects of LIF-STAT3 on ES cell proliferation (4). Thus it is apparent that for ES cells to remain in a proliferative state, the effects of various signaling pathways must be balanced. In this context, it is relevant to consider that the expression of transcription factors such as Oct-3/4 (a POU-family transcription factor initially identified in embryonic carcinoma cells) and Nanog (a homeobox transcription factor that plays a crucial role in the second embryonic cell fate specification following the formation of the blastocyst) are required to maintain the pluripotency of embryonic cells both in vitro and in vivo and can be considered as master regulators of ES cell pluripotency.

The generation of insulin-secreting cells from pluripotent embryonic stem cells is hampered by the fact that pancreatic β-cells appear late during embryonic development. Such an event may thus involve the sequential activation and deactivation of a considerable number of genes controlling first the generation of endoderm lineage cells and the subsequent generation of the precursors of pancreatic cells, the endocrine lineage cells, and finally the insulin-secreting cells. The knowledge of the developmental pathway and its key genetic steps leading from an undifferentiated endoderm to mature β-cells has been instrumental for designing in vitro β-cell differentiation protocols.

The derivation of insulin-producing cells from mouse ES (mES) cells has been accomplished following two different strategies. In the first one, the authors used a genetic cell-trapping approach (23) that basically consisted of the expres-
During the last four decades, several studies have indicated that the pancreas contains a pool of progenitor cells that have the potential for lineage-restricted differentiation and are capable of developing into a pancreatic phenotype. The data suggest that human and rodent pancreatic duct cells, islet-derived cells, and exocrine tissue have precursor cells that can differentiate toward β-cells. Thus it does seem that there is a regeneration of pancreatic β-cells, but it is not clear whether it happens by self-replication or neogenesis. In addition, up to the moment, the identity of the possible β-cell progenitor or stem cell is not clear, if indeed it exists. In fact, a recent genetic lineage study (5) claims that in adult mice, the replication of preexisting β-cells is the dominant pathway for the formation of new β-cells. On the other hand, a more recent study (20) shows a clonal isolation of multipotential precursor cells from mouse adult pancreas called pancreas-derived multipotent precursors. These precursor cells arise from single islet and ductal cells.

To date, the best candidates for adult pancreatic stem or progenitor cells are 1) duct cells; 2) exocrine tissue; 3) nestin-positive islet-derived progenitor cells; 4) neurogenin-3-positive cells (ngn-3 is a proendocrine β-helix-loop-helix transcription factor); 5) pancreas-derived multipotent precursors; and 6) mature β-cells. Regarding this, the first report to describe in vitro-generated insulin-producing isletlike clusters was based on the use of a reporter-selector gene controlled by the insulin promoter. In this report, mES cells were first forced to differentiate into embryoid bodies (EB) by being cultured in a medium lacking supplemental LIF. Then, for their final differentiation and maturation, the EB were cultured in the presence of nicotinamide and glucose, and those cells expressing the insulin gene were selected if they grew in the presence of neomycin. Using this approach, the authors isolated insulin-containing clones that displayed in vitro regulated hormone secretion and corrected hyperglycemia when transplanted into diabetic mice. The same group has recently described a modified procedure based on the use of the Nkx6.1 (which belongs to the NK-homeodomain protein-encoding genes with endocrine specification functions) gene promoter for cell trapping of neural stem cells. In this report, mES cells were first forced to differentiate into embryoid bodies (EB) by being cultured in a serum-free medium to select nestin-positive cells. Nestin is an intermediate filament protein expressed in neural stem cells. Then, these cells were expanded by culture in the presence of a fibroblast growth factor and differentiated into islet-like structures by supplementing the culture medium with B27 and nicotinamide.

With this procedure, cells that displayed regulated insulin secretion were obtained. However, the normalization of glycemia after injecting them into diabetic animals was not achieved.

The propagation of human ES (hES) cells requires feeder fibroblasts, because these cells are insensitive to LIF (11). The presence of insulin-positive cells during the spontaneous differentiation of EB, generated from hES cells, propagated on layers of mouse embryonic fibroblasts was first reported by Assady et al. (1). Following Lumelsky’s differentiation protocol, Seguev et al. (21) generated immature islet cell precursors from hES cell lines. These cells showed a response to nonmetabolizable secretagogues but had a poor glucose-induced insulin secretion. The fact that a significant amount of generated cell clusters was found to be positive for the three islet hormones raises the possibility that these cells resemble immature pancreatic cells. The concept that mature endocrine pancreatic cells arise directly from a common precursor cell type has been challenged recently (9). Thus there is a possibility that cultured cells concentrate these hormones from the surrounding culture media (17). As outlined in this review, a directed differentiation of mouse and human ES cells toward insulin-producing cells has been reported. Murine insulin-producing cells derived from ES cells are able to normalize glycemia in diabetic animals. Until now, this fundamental piece of evidence still has to be found in human cell studies and reflects a lack of functional in vivo response. The development of protocols for the generation of mature β-cell-like structures from immature insulin-producing human cells may be helpful to overcome this obstacle. The cell-trapping approach used by the authors for the differentiation of insulin-producing cells from embryonic cells in mice could be instrumental in this aspect (23, 15).
on the expansion of mouse pancreatic duct cells (18). Afterwards, Bonner-Weir et al. (3) generated the same type of insulin-producing isletlike clusters from cultivated islet buds developed from human pancreatic duct cells in vitro. These data provide evidence of the potential to expand and differentiate duct cells to islet cells. However, at this time, the expansion capacity of these cultivated cells is limited, and from the clinical point of view, protocols for in vitro amplification need further optimization to produce a sufficient number of fully differentiated cells to allow a successful transplantation. Finally, a very recent report (2) describes the generation of insulin-producing cells from pancreatic exocrine tissue. Both exocrine and endocrine pancreas originate from a domain of the foregut endoderm, which expresses at early developmental stages the pancreatic duodenal homeobox factor (Pdx-1). The targeted inactivation of this gene leads to an anaplastic phenotype, demonstrating its major role in both exocrine and endocrine pancreatic development. In addition, signaling induced by soluble factors is a prerequisite to pancreatic lineage specification and triggers the emergence of pancreatic precursors expressing Pdx-1. Moreover, as Baeyens et al. (2) indicate, there are data suggesting the in vivo existence of acinar-islet transitional cells and the “spontaneous” transdifferentiation of acinar cells to insulin-expressing cells. Altogether, this may suggest that a population of acinar cells, in the presence of certain soluble factors, is competent to adopt an endocrine fate. Concerning nestin-positive cells, some reports suggest that pancreatic precursor cells express nestin (25), an intermediate filament protein that is a marker of neural stem cells. These nestin-positive islet-derived progenitor cells also express insulin, glucagon, and Pdx-1 as well as low levels of insulin secretion. However, other studies suggest that nestin expression is not related to pancreatic precursor identity. Recent data indicate that Ngn-3-positive cells are endocrine progenitors both in the adult pancreas and in the embryo and that Ngn-3 expression is not seen outside the islets (8). Nevertheless, low levels of Ngn-3 expression within a population of duct cells are not excluded by these studies.

The identification and full characterization of putative stem cells from adult tissues, together with the development of in vitro expansion and differentiation protocols, will be crucial for exploiting these cells in diabetes cell therapy. Given the differences between rodents and humans, it is anticipated that more research will have to be done with human stem cells to understand B-cell regeneration and neogenesis.

OTHER SOURCES OF ADULT STEM CELLS

The existence of extrapancreatic progenitor cells that differentiate into B-cells has been suggested in several reports. Accordingly, due to the common endodermic origin of the liver and pancreas, the liver was one of the first places examined to find these cells. Rodent-liver stem cells (7) and human fetal liver epithelial progenitor cells (24) have been differentiated in vitro into insulin-secreting cells. In the first study, an adenovirus-mediated delivery of CMV-promoter-driven Pdx-1 genes to the liver of streptozotocin-treated diabetic mice led to the appearance of insulin in the liver and an improvement of the hyperglycemia. The report of Zalzman et al. (24) showed that the expression of Pdx-1 in fetal human progenitor liver cells induced the activation of several B-cell genes, produced and released insulin in response to glucose, and, when transplanted into hyperglycemic immunodeficient mice, these cells restored and maintained normoglycemia for long periods. In addition, these cells that possess a high replication capacity were further immortalized by introducing the gene for the catalytic subunit of human telomerase. Thus, in the liver, the origin of these insulin-producing cells can be 1) pluripotent liver precursor cells, 2) the transdifferentiation of a limited number of hepatocytes, and 3) circulating stem cells that reach the liver. Another tissue with similar embryological origins is the upper gastrointestinal tract. In a recent study, Koijima et al. (14) transfected rat intestinal stem cells with genes encoding Pdx-1 and Isl-1 and exposed them to betacellulin. The resultant cells produced insulin and reduced plasma glucose levels after transplantation in diabetic rats.

To this point, the question that arises is whether adult progenitor cells retaining sufficient multipotency to generate pancreatic endocrine cells are present in other tissues. It has been shown that mouse bone marrow contains adult stem cells with a broad differentiation potential. In addition, there is considerable evidence indicating that bone marrow harbors cells that can populate adult organs. In this sense, there exists a population of cells from the adult bone marrow called multipotent adult progenitor cells (MAPCs) that have the potential to differentiate into ectodermal, mesodermal, and endodermal cell types “in vitro” and “in vivo”. Until now, the differentiation of MAPCs into pancreatic endocrine cells has not yet been shown, but their high plasticity makes it possible to generate islet cells from them. Finally, two recent studies indicate that transplanted bone marrow-derived stem cells can generate functional pancreatic endocrine cells. The study of Janus et al. (12) reports that the transplantation of bone marrow cells that selectively express the enhanced green fluorescent protein (EGFP) if the insulin gene is actively transcribed into lethally irradiated recipient mice gives rise to EGFP-positive insulin-producing cells in pancreatic islets. These bone marrow-derived cells expressed genetic markers of B-cells and exhibited glucose-dependent insulin secretion. There was no evidence of cell fusion in this study. However, the proportion of EGFP-expressing cells within the islet was up to 3% of the total number of islet insulin-positive cells. Similar experiments have been done in streptozotocin diabetic mice, where Hess et al. (10) have shown that the transplantation of adult bone marrow-derived cells reduced hyperglycemia in the diabetic animals. The mechanisms proposed are 1) the differentiation of marrow-derived cells into insulin-expressing cells takes place in the islets and ductus and 2) transplanted bone marrow-derived stem cells initiate endogenous pancreatic tissue regeneration. Finally, in a more recent study, the authors exploited the unexpected plasticity of monocytes to derive insulin-producing cells from human peripheral blood (19). In this study, human peripheral blood monocytes were treated with the macrophage colony-stimulating factor and IL-3 for 6 days and then cultured for 4–8 days in the presence of the epidermal growth factor, hepatic growth factor, and nicotinamide. In these conditions, neoislets were generated from peripheral blood monocytes that had glucose-regulated insulin secretion. Autologous transplantation may help to overcome rejection in certain forms of diabetes such as type 2 and monogenic forms of diabetes mellitus. However, type 1 diabetes, being an autoimmune disease, will need additional strategies.
In conclusion, future sources of β-cells derived from embryonic or adult stem cells offer an important potential on the road ahead to provide a cure for diabetes. At the moment, it is difficult to know which source of stem cells has the greatest potential. The β-cell is a very complex and differentiated cell. Thus adult stem cells, particularly those coming from the pancreas, seem to be easier to fully differentiate in normal β-cells with precise glucose recognition and regulated insulin secretion. In contrast, embryonic stem cells are more difficult to differentiate, but proliferation is not a major problem. In addition, regardless of the origin of newly generated β-cells, these cells will need to function in other places besides the pancreas and will need to be protected from rejection and autoimmune destruction.

Thus research on both adult and embryonic stem cells should be pursued, because embryonic stem cells will be crucial to improve the research in adult stem cells and vice versa. Although stem cell research is on the cutting edge of biological science, it is still just beginning. Before the enormous potential and capabilities of stem cells can be developed for the treatment of diabetes, several goals need to be achieved by stem cell physiologists such as the establishment of the physiological basis for stem cell reprogramming, proliferation, differentiation, and self-renewal along with techniques to propagate them reliably and a consensus on the physiological criteria confirming the restoration of the tissue function following stem cell transplantation.

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