

Stem Cells to Pancreatic Islet, Insulin Secreting Cells

Stem cells are receiving considerable attention because of the ethical issues they raise and the potential they offer for treatment of many human diseases. The recent report that stem cells can be cajoled to produce insulin may raise the debate to a new level.

The endocrine pancreas (islets of Langerhans) contains 4 cell types that secrete peptide hormones: insulin (β cells), glucagon (α cells), somatostatin (δ cells), and pancreatic polypeptide (PP cells). Because of the close association of these cells with neural cells in the pancreas and their similar embryonic origins, the National Institutes of Health group headed by McKay postulated that experimental strategies that induce embryonic stem cells (ES cells) to become neural cells could be used to induce ES cells to become pancreatic endocrine cells.

Using techniques previously developed, they first induced ES cells to differentiate as neural precursor cells. These cells expressed a marker gene, termed nestin. Their protocol then progressed through a series of steps that sequentially expanded and selected pancreatic endocrine progenitor cells, using various markers to identify these cells and their precursors. By the end of the protocol, which took approximately 3 weeks, they generated relatively large numbers of insulin-positive cells, which resided in clusters in close association with neurons. Confocal microscopy revealed that the insulin-positive cells were located in the centers of the clusters surrounded by neurons. Immunostaining revealed that glucagon, somatostatin, and pancreatic polypeptide also were produced by cells in the clusters that tended to surround the insulin-positive cells. Pancreatic exocrine markers were not detected. Thus, the ES cells generated multicellular structures that resembled pancreatic islets *in vivo*.

The investigators next performed clonal analysis to determine if the islet-like cells and the neurons developed from independent progenitor cells or from a common progenitor cell. The results suggested they arose from a common progenitor cell pool.

Experiments were next carried out to show that the islet-like cells release insulin in response to glucose in a dose-dependent manner with kinetics similar to those of pancreatic islet cells in culture. Quantitation revealed that the ES cell-derived cells contained about 1/50th the amount of insulin that normal islet cells contain. The researchers then examined the effect of several known agonists and antagonists of insulin secretion on insulin release. All of the agents tested produced appropriate responses, indicating that the machinery used to regulate insulin secretion by islet cells is present in the islet-like cells.

Finally, the authors tested the ability of the ES cell-derived clusters to survive and function *in vivo* by grafting the cell clusters subcutaneously into the shoulders of streptozocin-diabetic mice. When harvested later, the clusters were shown to vascularize and to remain insulin-reactive. Although grafted mice were able to maintain body weight and survive longer than sham-grafted controls, they were not able to sustain normal blood glucose levels, which the authors attributed to

the relatively low levels of insulin per cell of the ES cell-derived islet-like cells.

The researchers concluded that engineering of ES cells to produce an abundant source of immunocompatible tissue for transplantation holds considerable promise as a future strategy for treating diabetes. In an accompanying commentary, Vogel points out that although others have reported promising results in transplanting pancreatic cells from cadavers into diabetic patients, the demand for cells is far greater than the current supply.

Lumelsky N, et al. Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science* 2001;292:1389-1394.

Vogel G. Stem cells are coaxed to produce insulin. *Science* 2001;292:615-617.

Editor's comment: *As the authors acknowledge, these very promising results are still only preliminary. Nevertheless, they have caused excitement in the scientific community. It will be interesting to see if human ES cells behave like the mouse ES cells. If so, it will add considerable fuel to the debate over the use of ES cells to treat human disease.*

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Figure
Turning Mouse Embryonic Stem (ES) Cells Into
Insulin-Secreting "Islet Clusters"

Stage 1 (2-3 days):
Expand ES cells in the presence
of leukemia inhibitory factor (LIF);



Stage 2 (4 days):
Removing LIF prompts disorganized
clumps of differentiating cells
(called embryoid bodies) to form.



Stage 3 (6-7 days):
Growing embryoid bodies in
serum-free medium kills many cells;
nestin-positive cells remain.



Stage 4 (6 days):
Nestin-positive cells exposed to basic
fibroblast growth factor (bFGF)
and several other proteins become
pancreatic precursor cells.



Stage 5 (6 days):
Removing bFGF causes some cells
to differentiate into insulin-secreting
clusters of cells resembling
pancreatic islets.



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