Diabetes exacts its toll on many Americans, young and old. For years, researchers have painstakingly dissected this complicated disease caused by the destruction of insulin producing islet cells of the pancreas. Despite progress in understanding the underlying disease mechanisms for diabetes, there is still a paucity of effective therapies. For years investigators have been making slow, but steady, progress on experimental strategies for pancreatic transplantation and islet cell replacement. Now, researchers have turned their attention to adult stem cells that appear to be precursors to islet cells and embryonic stem cells that produce insulin.

INTRODUCTION

For decades, diabetes researchers have been searching for ways to replace the insulin-producing cells of the pancreas that are destroyed by a patient’s own immune system. Now it appears that this may be possible. Each year, diabetes affects more people and causes more deaths than breast cancer and AIDS combined. Diabetes is the seventh leading cause of death in the United States today, with nearly 200,000 deaths reported each year. The American Diabetes Association estimates that nearly 16 million people, or 5.9 percent of the United States population, currently have diabetes.

Diabetes is actually a group of diseases characterized by abnormally high levels of the sugar glucose in the bloodstream. This excess glucose is responsible for most of the complications of diabetes, which include blindness, kidney failure, heart disease, stroke, neuropathy, and amputations. Type 1 diabetes, also known as juvenile-onset diabetes, typically affects children and young adults. Diabetes develops when the body’s immune system sees its own cells as foreign and attacks and destroys them. As a result, the islet cells of the pancreas, which normally produce insulin, are destroyed. In the absence of insulin, glucose cannot enter the cell and glucose accumulates in the blood. Type 2 diabetes, also called adult-onset diabetes, tends to affect older, sedentary, and overweight individuals with a family history of diabetes. Type 2 diabetes occurs when the body cannot use insulin effectively. This is called insulin resistance and the result is the same as with type 1 diabetes—a build up of glucose in the blood.

There is currently no cure for diabetes. People with type 1 diabetes must take insulin several times a day and test their blood glucose concentration three to four times a day throughout their entire lives. Frequent monitoring is important because patients who keep their blood glucose concentrations as close to normal as possible can significantly reduce many of the complications of diabetes, such as retinopathy (a disease of the small blood vessels of the eye which can lead to blindness) and heart disease, that tend to develop over time. People with type 2 diabetes can often control their blood glucose concentrations through a combination of diet, exercise, and oral medication. Type 2 diabetes often progresses to the point where only insulin therapy will control blood glucose concentrations.

Each year, approximately 1,300 people with type 1 diabetes receive whole-organ pancreas transplants. After a year, 83 percent of these patients, on average, have no symptoms of diabetes and do not have to take insulin to maintain normal glucose concentrations in the blood. However, the demand for transplantable pancreases outweighs their availability. To prevent the body from rejecting the transplanted pancreas, patients must take powerful drugs that suppress the immune system for their entire lives, a regimen that makes them susceptible to a
host of other diseases. Many hospitals will not perform a pancreas transplant unless the patient also needs a kidney transplant. That is because the risk of infection due to immunosuppressant therapy can be a greater health threat than the diabetes itself. But if a patient is also receiving a new kidney and will require immunosuppressant drugs anyway, many hospitals will perform the pancreas transplant.

Over the past several years, doctors have attempted to cure diabetes by injecting patients with pancreatic islet cells—the cells of the pancreas that secrete insulin and other hormones. However, the requirement for steroid immunosuppressant therapy to prevent rejection of the cells increases the metabolic demand on insulin-producing cells and eventually they may exhaust their capacity to produce insulin. The deleterious effect of steroids is greater for islet cell transplants than for whole-organ transplants. As a result, less than 8 percent of islet cell transplants performed before last year had been successful.

More recently, James Shapiro and his colleagues in Edmonton, Alberta, Canada, have developed an experimental protocol for transplanting islet cells that involves using a much larger amount of islet cells and a different type of immunosuppressant therapy. In a recent study, they report that [17], seven of seven patients who received islet cell transplants no longer needed to take insulin, and their blood glucose concentrations were normal a year after surgery. The success of the Edmonton protocol is now being tested at 10 centers around the world.

If the success of the Edmonton protocol can be duplicated, many hurdles still remain in using this approach on a wide scale to treat diabetes. First, donor tissue is not readily available. Islet cells used in transplants are obtained from cadavers, and the procedure requires at least two cadavers per transplant. The islet cells must be immunologically compatible, and the tissue must be freshly obtained—within eight hours of death. Because of the shortage of organ donors, these requirements are difficult to meet and the waiting list is expected to far exceed available tissue, especially if the procedure becomes widely accepted and available. Further, islet cell transplant recipients face a lifetime of immunosuppressant therapy, which makes them susceptible to other serious infections and diseases.

DEVELOPMENT OF THE PANCREAS

Before discussing cell-based therapies for diabetes, it is important to understand how the pancreas develops. In mammals, the pancreas contains three classes of cell types: the ductal cells, the acinar cells, and the endocrine cells. The endocrine cells produce the hormones glucagon, somatostatin, pancreatic polypeptide (PP), and insulin, which are secreted into the bloodstream and help the body regulate sugar metabolism. The acinar cells are part of the exocrine system, which manufactures digestive enzymes, and ductal cells from the pancreatic ducts, which connect the acinar cells to digestive organs.

In humans, the pancreas develops as an outgrowth of the duodenum, a part of the small intestine. The cells of both the exocrine system—the acinar cells—and of the endocrine system—the islet cells—seem to originate from the ductal cells during development. During development these endocrine cells emerge from the pancreatic ducts and form aggregates that eventually form what is known as Islets of Langerhans. In humans, there are four types of islet cells: the insulin-producing beta cells; the alpha cells, which produce glucagon; the delta cells, which secrete somatostatin; and the PP-cells, which produce pancreatic polypeptide. The hormones released from each type of islet cell have a role in regulating hormones released from other islet cells. In the human pancreas, 65 to 90 percent of islet cells are beta cells, 15 to 20 percent are alpha-cells, 3 to 10 percent are delta cells, and one percent is PP cells. Acinar cells form small lobules contiguous with the ducts (see Figure 7.1. Insulin Production in the Human Pancreas). The resulting pancreas is a combination of a lobulated, branched acinar gland that forms the exocrine pancreas, and, embedded in the acinar gland, the islets of Langerhans, which constitute the endocrine pancreas.

During fetal development, new endocrine cells appear to arise from progenitor cells in the pancreatic ducts. Many researchers maintain that some sort of islet stem cell can be found intermingled with ductal cells during fetal development and that these stem cells give rise to new endocrine cells as the fetus develops. Ductal cells can be distinguished from endocrine cells by their structure and by the genes they express. For example, ductal cells typically express a gene known as cytokeratin-9.
Figure 7.1. Insulin Production in the Human Pancreas.
The pancreas is located in the abdomen, adjacent to the duodenum (the first portion of the small intestine). A cross-section of the pancreas shows the islet of Langerhans which is the functional unit of the endocrine pancreas. Encircled is the beta cell that synthesizes and secretes insulin. Beta cells are located adjacent to blood vessels and can easily respond to changes in blood glucose concentration by adjusting insulin production. Insulin facilitates uptake of glucose, the main fuel source, into cells of tissues such as muscle.
Stem Cells and Diabetes

(CK-9), which encodes a structural protein. Beta islet cells, on the other hand, express a gene called PDX-1, which encodes a protein that initiates transcription from the insulin gene. These genes, called cell markers, are useful in identifying particular cell types.

Following birth and into adulthood, the source of new islet cells is not clear, and some controversy exists over whether adult stem cells exist in the pancreas. Some researchers believe that islet stem cell-like cells can be found in the pancreatic ducts and even in the islets themselves. Others maintain that the ductal cells can differentiate into islet precursor cells, while others hold that new islet cells arise from stem cells in the blood. Researchers are using several approaches for isolating and cultivating stem cells or islet precursor cells from fetal and adult pancreatic tissue. In addition, several new promising studies indicate that insulin-producing cells can be cultivated from embryonic stem cell lines.

DEVELOPMENT OF CELL-BASED THERAPIES FOR DIABETES

In developing a potential therapy for patients with diabetes, researchers hope to develop a system that meets several criteria. Ideally, stem cells should be able to multiply in culture and reproduce themselves exactly. That is, the cells should be self-renewing. Stem cells should also be able to differentiate in vivo to produce the desired kind of cell. For diabetes therapy, it is not clear whether it will be desirable to produce only beta cells—the islet cells that manufacture insulin—or whether other types of pancreatic islet cells are also necessary. Studies by Bernat Soria and colleagues, for example, indicate that isolated beta cells—those cultured in the absence of the other types of islet cells—are less responsive to changes in glucose concentration than intact islet clusters made up of all islet cell types. Islet cell clusters typically respond to higher-than-normal concentrations of glucose by releasing insulin in two phases: a quick release of high concentrations of insulin and a slower release of lower concentrations of insulin. In this manner the beta cells can fine-tune their response to glucose. Extremely high concentrations of glucose may require that more insulin be released quickly, while intermediate concentrations of glucose can be handled by a balance of quickly and slowly released insulin.

Isolated beta cells, as well as islet clusters with lower-than-normal amounts of non-beta cells, do not release insulin in this biphasic manner. Instead insulin is released in an all-or-nothing manner, with no fine-tuning for intermediate concentrations of glucose in the blood [5, 18]. Therefore, many researchers believe that it will be preferable to develop a system in which stem or precursor cell types can be cultured to produce all the cells of the islet cluster in order to generate a population of cells that will be able to coordinate the release of the appropriate amount of insulin to the physiologically relevant concentrations of glucose in the blood.

FETAL TISSUE AS SOURCE FOR ISLET CELLS

Several groups of researchers are investigating the use of fetal tissue as a potential source of islet progenitor cells. For example, using mice, researchers have compared the insulin content of implants from several sources of stem cells—fresh human fetal pancreatic tissue, purified human islets, and cultured islet tissue [2]. They found that insulin content was initially higher in the fresh tissue and purified islets. However, with time, insulin concentration decreased in the whole tissue grafts, while it remained the same in the purified islet grafts. When cultured islets were implanted, however, their insulin content increased over the course of three months. The researchers concluded that precursor cells within the cultured islets were able to proliferate (continue to replicate) and differentiate (specialize) into functioning islet tissue, but that the purified islet cells (already differentiated) could not further proliferate when grafted. Importantly, the researchers found, however, that it was also difficult to expand cultures of fetal islet progenitor cells in culture [7].

ADULT TISSUE AS SOURCE FOR ISLET CELLS

Many researchers have focused on culturing islet cells from human adult cadavers for use in developing transplantable material. Although differentiated beta cells are difficult to proliferate and culture, some researchers have had success in engineering such cells to do this. For example, Fred Levine and his colleagues at the University of California, San Diego, have engineered islet cells isolated from human cadavers by adding to the cells’ DNA special genes...
that stimulate cell proliferation. However, because once such cell lines that can proliferate in culture are established, they no longer produce insulin. The cell lines are further engineered to express the beta islet cell gene, PDX-1, which stimulates the expression of the insulin gene. Such cell lines have been shown to propagate in culture and can be induced to differentiate to cells, which produce insulin. When transplanted into immune-deficient mice, the cells secrete insulin in response to glucose. The researchers are currently investigating whether these cells will reverse diabetes in an experimental diabetes model in mice [6, 8].

These investigators report that these cells do not produce as much insulin as normal islets, but it is within an order of magnitude. The major problem in dealing with these cells is maintaining the delicate balance between growth and differentiation. Cells that proliferate well do not produce insulin efficiently, and those that do produce insulin do not proliferate well. According to the researchers, the major issue is developing the technology to be able to grow large numbers of these cells that will reproducibly produce normal amounts of insulin [9].

Another promising source of islet progenitor cells lies in the cells that line the pancreatic ducts. Some researchers believe that multipotent (capable of forming cells from more than one germ layer) stem cells are intermingled with mature, differentiated duct cells, while others believe that the duct cells themselves can undergo a differentiation, or a reversal to a less mature type of cell, which can then differentiate into an insulin-producing islet cell. Susan Bonner-Weir and her colleagues reported last year that when ductal cells isolated from adult human pancreatic tissue were cultured, they could be induced to differentiate into clusters that contained both ductal and endocrine cells. Over the course of three to four weeks in culture, the cells secreted low amounts of insulin when exposed to low concentrations of glucose, and higher amounts of insulin when exposed to higher glucose concentrations. The researchers have determined by immunochemistry and ultrastructural analysis that these clusters contain all of the endocrine cells of the islet [4].

Bonner-Weir and her colleagues are working with primary cell cultures from duct cells and have not established cell lines that can grow indefinitely. However the cells can be expanded. According to the researchers, it might be possible in principle to do a biopsy and remove duct cells from a patient and then proliferate the cells in culture and give the patient back his or her own islets. This would work with patients who have type 1 diabetes and who lack functioning beta cells, but their duct cells remain intact. However, the autoimmune destruction would still be a problem and potentially lead to destruction of these transplanted cells [3]. Type 2 diabetes patients might benefit from the transplantation of cells expanded from their own duct cells since they would not need any immunosuppression. However, many researchers believe that if there is a genetic component to the death of beta cells, then beta cells derived from ductal cells of the same individual would also be susceptible to autoimmune attack.

Some researchers question whether the ductal cells are indeed undergoing a dedifferentiation or whether a subset of stem-like or islet progenitors populate the pancreatic ducts and may be co-cultured along with the ductal cells. If ductal cells die off but islet precursors proliferate, it is possible that the islet precursor cells may overtake the ductal cells in culture and make it appear that the ductal cells are dedifferentiating into stem cells. According to Bonner-Weir, both dedifferentiated ductal cells and islet progenitor cells may occur in pancreatic ducts.

Ammon Peck of the University of Florida, Vijayakumar Ramiya of Ixion Biotechnology in Alachua, FL, and their colleagues [13, 14] have also cultured cells from the pancreatic ducts from both humans and mice. Last year, they reported that pancreatic ductal epithelial cells from adult mice could be cultured to yield islet-like structures similar to the cluster of cells found by Bonner-Weir. Using a host of islet-cell markers they identified cells that produced insulin, glucagon, somatostatin, and pancreatic polypeptide. When the cells were implanted into diabetic mice, the diabetes was reversed.

Joel Habener has also looked for islet-like stem cells from adult pancreatic tissue. He and his colleagues have discovered a population of stem-like cells within both the adult pancreas islets and pancreatic ducts. These cells do not express the marker typical of ductal cells, so they are unlikely to be ductal cells, according to Habener. Instead, they express a marker called nestin, which is typically found in developing
neural cells. The nestin-positive cells do not express markers typically found in mature islet cells. However, depending upon the growth factors added, the cells can differentiate into different types of cells, including liver, neural, exocrine pancreas, and endocrine pancreas, judged by the markers they express, and can be maintained in culture for up to eight months [20].

**EMBRYONIC STEM CELLS**

The discovery of methods to isolate and grow human embryonic stem cells in 1998 renewed the hopes of doctors, researchers, and diabetes patients and their families that a cure for type 1 diabetes, and perhaps type 2 diabetes as well, may be within striking distance. In theory, embryonic stem cells could be cultivated and coaxed into developing into the insulin-producing islet cells of the pancreas. With a ready supply of cultured stem cells at hand, the theory is that a line of embryonic stem cells could be grown up as needed for anyone requiring a transplant. The cells could be engineered to avoid immune rejection. Before transplantation, they could be placed into nonimmunogenic material so that they would not be rejected and the patient would avoid the devastating effects of immunosuppressant drugs. There is also some evidence that differentiated cells derived from embryonic stem cells might be less likely to cause immune rejection (see Chapter 10. Assessing Human Stem Cell Safety). Although having a replenishable supply of insulin-producing cells for transplant into humans may be a long way off, researchers have been making remarkable progress in their quest for it. While some researchers have pursued the research on embryonic stem cells, other researchers have focused on insulin-producing precursor cells that occur naturally in adult and fetal tissues.

Since their discovery three years ago, several teams of researchers have been investigating the possibility that human embryonic stem cells could be developed as a therapy for treating diabetes. Recent studies in mice show that embryonic stem cells can be coaxed into differentiating into insulin-producing beta cells, and new reports indicate that this strategy may be possible using human embryonic cells as well.

Last year, researchers in Spain reported using mouse embryonic stem cells that were engineered to allow researchers to select for cells that were differentiating into insulin-producing cells [19]. Bernat Soria and his colleagues at the Universidad Miguel Hernandez in San Juan, Alicante, Spain, added DNA containing part of the insulin gene to embryonic cells from mice. The insulin gene was linked to another gene that rendered the mice resistant to an antibiotic drug. By growing the cells in the presence of an antibiotic, only those cells that were activating the insulin promoter were able to survive. The cells were cloned and then cultured under varying conditions. Cells cultured in the presence of low concentrations of glucose differentiated and were able to respond to changes in glucose concentration by increasing insulin secretion nearly sevenfold. The researchers then implanted the cells into the spleens of diabetic mice and found that symptoms of diabetes were reversed.

Manfred Ruediger of Cardion, Inc., in Erkrath, Germany, is using the approach developed by Soria and his colleagues to develop insulin-producing human cells derived from embryonic stem cells. By using this method, the non-insulin-producing cells will be killed off and only insulin-producing cells should survive. This is important in ensuring that undifferentiated cells are not implanted that could give rise to tumors [15]. However, some researchers believe that it will be important to engineer systems in which all the components of a functioning pancreatic islet are allowed to develop.

Recently Ron McKay and his colleagues described a series of experiments in which they induced mouse embryonic cells to differentiate into insulin-secreting structures that resembled pancreatic islets [10]. McKay and his colleagues started with embryonic stem cells and let them form embryoid bodies—an aggregate of cells containing all three embryonic germ layers. They then selected a population of cells from the embryoid bodies that expressed the neural marker nestin (see Appendix B. Mouse Embryonic Stem Cells). Using a sophisticated five-stage culturing technique, the researchers were able to induce the cells to form islet-like clusters that resembled those found in native pancreatic islets. The cells responded to normal glucose concentrations by secreting insulin, although insulin amounts were lower than those secreted by normal islet cells (see Figure 7.2. Development of Insulin-Secreting Pancreatic-Like Cells From Mouse Embryonic Stem Cells). When the cells were injected into diabetic mice, they
Mouse embryonic stem cells were derived from the inner cell mass of the early embryo (blastocyst) and cultured under specific conditions. The embryonic stem cells (in blue) were then expanded and differentiated. Cells with markers consistent with islet cells were selected for further differentiation and characterization. When these cells (in purple) were grown in culture, they spontaneously formed three-dimensional clusters similar in structure to normal pancreatic islets. The cells produced and secreted insulin. As depicted in the chart, the pancreatic islet-like cells showed an increase in release of insulin as the glucose concentration of the culture media was increased. When the pancreatic islet-like cells were implanted in the shoulder of diabetic mice, the cells became vascularized, synthesized insulin, and maintained physical characteristics similar to pancreatic islets.
survived, although they did not reverse the symptoms of diabetes.

According to McKay, this system is unique in that the embryonic cells form a functioning pancreatic islet, complete with all the major cell types. The cells assemble into islet-like structures that contain another layer, which contains neurons and is similar to intact islets from the pancreas [11]. Several research groups are trying to apply McKay’s results with mice to induce human embryonic stem cells to differentiate into insulin-producing islets.

Recent research has also provided more evidence that human embryonic cells can develop into cells that can and do produce insulin. Last year, Melton, Nissim Benvinisty of the Hebrew University in Jerusalem, and Josef Itskovitz-Eldor of the Technion in Haifa, Israel, reported that human embryonic stem cells could be manipulated in culture to express the *PDX-1* gene, a gene that controls insulin transcription [16]. In these experiments, researchers cultured human embryonic stem cells and allowed them to spontaneously form embryoid bodies (clumps of embryonic stem cells composed of many types of cells from all three germ layers). The embryoid bodies were then treated with various growth factors, including nerve growth factor. The researchers found that both untreated embryoid bodies and those treated with nerve growth factor expressed *PDX-1*. Embryonic stem cells prior to formation of the aggregated embryoid bodies did not express *PDX-1*. Because expression of the *PDX-1* gene is associated with the formation of beta islet cells, these results suggest that beta islet cells may be one of the cell types that spontaneously differentiate in the embryoid bodies. The researchers now think that nerve growth factor may be one of the key signals for inducing the differentiation of beta islet cells and can be exploited to direct differentiation in the laboratory. Complementing these findings is work done by Jon Odorico of the University of Wisconsin in Madison using human embryonic cells of the same source. In preliminary findings, he has shown that human embryonic stem cells can differentiate and express the insulin gene [12].

More recently, Itskovitz-Eldor and his Technion colleagues further characterized insulin-producing cells in embryoid bodies [1]. The researchers found that embryonic stem cells that were allowed to spontaneously form embryoid bodies contained a significant percentage of cells that express insulin. Based on the binding of antibodies to the insulin protein, Itskovitz-Eldor estimates that 1 to 3 percent of the cells in embryoid bodies are insulin-producing beta-islet cells. The researchers also found that cells in the embryoid bodies express glut-2 and islet-specific glucokinase, genes important for beta cell function and insulin secretion. Although the researchers did not measure a time-dependent response to glucose, they did find that cells cultured in the presence of glucose secrete insulin into the culture medium. The researchers concluded that embryoid bodies contain a subset of cells that appear to function as beta cells and that the refining of culture conditions may soon yield a viable method for inducing the differentiation of beta cells and, possibly, pancreatic islets.

Taken together, these results indicate that the development of a human embryonic stem cell system that can be coaxed into differentiating into functioning insulin-producing islets may soon be possible.

**FUTURE DIRECTIONS**

Ultimately, type 1 diabetes may prove to be especially difficult to cure, because the cells are destroyed when the body’s own immune system attacks and destroys them. This autoimmunity must be overcome if researchers hope to use transplanted cells to replace the damaged ones. Many researchers believe that at least initially, immunosuppressive therapy similar to that used in the Edmonton protocol will be beneficial. A potential advantage of embryonic cells is that, in theory, they could be engineered to express the appropriate genes that would allow them to escape or reduce detection by the immune system. Others have suggested that a technology should be developed to encapsulate or embed islet cells derived from islet stem or progenitor cells in a material that would allow small molecules such as insulin to pass through freely, but would not allow interactions between the islet cells and cells of the immune system. Such encapsulated cells could secrete insulin into the blood stream, but remain inaccessible to the immune system.

Before any cell-based therapy to treat diabetes makes it to the clinic, many safety issues must be addressed (see Chapter 10. Assessing Human Stem Cell Safety). A major consideration is whether any
precursor or stem-like cells transplanted into the body might revert to a more pluripotent state and induce the formation of tumors. These risks would seemingly be lessened if fully differentiated cells are used in transplantation.

But before any kind of human islet-precursor cells can be used therapeutically, a renewable source of human stem cells must be developed. Although many progenitor cells have been identified in adult tissue, few of these cells can be cultured for multiple generations. Embryonic stem cells show the greatest promise for generating cell lines that will be free of contaminants and that can self renew. However, most researchers agree that until a therapeutically useful source of human islet cells is developed, all avenues of research should be exhaustively investigated, including both adult and embryonic sources of tissue.

REFERENCES


11. McKay, R., personal communication


15. Ruediger, M., personal communication.


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