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Stem Cell Science:

Overviews of Selected Disease Areas

Type 1 Diabetes

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The Harvard Stem Cell Institute (HSCI) is a scientific collaborative established in 2004 to fulfill the promise of stem cell biology as the basis for the cure and treatment of a wide range of chronic diseases and medical conditions. HSCI's unique effort unites experts across the disciplines, schools and departments of Harvard University and all its affiliated research hospitals.

HSCI also sponsors public education programs concerning the scientific, legal and ethical implications of stem cell research, conducts a summer research program for college students, and helps educate high school teachers about stem cell science. HSCI depends upon the vision and generosity of private individuals, foundation and corporate donors to carry on its work, due to current U.S. restrictions on federal funding of embryonic stem cell research.

Introduction

The following scientific overview focuses on the use of stem cells-both in research and potential therapeutic applications - to address one of the most challenging, life-changing diseases and conditions of our time. This overview along with its companion pieces has two educational objectives: to make clear the opportunities and promise inherent in the basic science and to provide you with a picture of the research avenues that must be pursued to reach clinical applications. The overviews point to the areas where fundamental questions exist, where therapies need improvement, and where funding for research is urgently required.

Immense hope and scientific effort at institutions like the Harvard Stem Cell Institute have been invested in stem cells. Researchers seek the fullest understanding of how cells' fates are determined. They want to know how embryonic stem (ESC) cells recognize and respond to the signals that move them to become the full range of mature cells in an animal. They want to know how adult stem cells, whose fates are more restricted, contribute to the repair and regeneration of organs and tissues. Studying both types of cells in parallel will provide us information pertinent to developing stem cell therapies. With each experiment and clinical trial, researchers and clinicians move closer to these goals. This set of papers summarizes the current state of stem cell science in several key disease areas.

Stem cell research adds its own unique challenges to those faced by any early stage science. In fact, there are many cells, along with their behavior, that are still being discovered. In some cases the benefit, whether detailed fundamental knowledge, development of new drugs, or transplantable cells, is likely to be far in the future; in other cases, progress will come amazingly quickly. In stem cell research, as in few other contemporary scientific enterprises, success depends on supportive collaboration among diverse constituents: scientists and clinicians; local, state, and federal governments; universities and industry; and patients and their advocates. As a cross-institutional collaborative research and educational organization, HSCI is committed to meeting this challenge. We hope these disease overviews will help you better understand the research areas that matter to you.

I would welcome your thoughts about these overviews, your questions, and concerns. Contact me at brock_reeve@harvard.edu.

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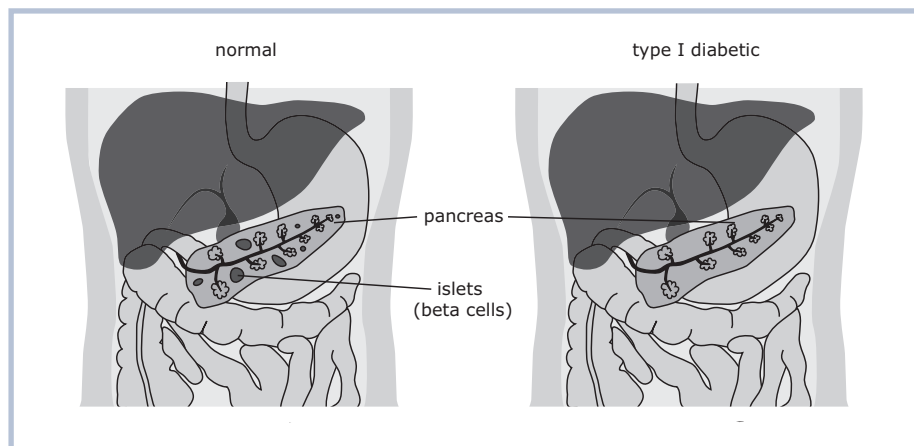
Type 1 Diabetes

A cell therapy treatment for type 1 diabetes requires the replacement of missing beta cells with new ones.

The American Diabetes Association estimates that 5 to 10 percent of Americans diagnosed with this disease have type 1 diabetes. Type 1 diabetes, also called juvenile diabetes, is an autoimmune disease that frequently presents itself from infancy to the late 30s. It results from the body's failure to produce insulin, a hormone that signals the body to allow glucose to enter cells and fuel them. The fundamental cause of type 1 diabetes is the absence of a single kind of cell, the pancreatic beta islet cell (beta cell). For unknown reasons, the immune system sometimes launches an attack against the body's own insulin-producing beta cells and destroys them. The current treatment for type 1 diabetes calls for injections of insulin to control blood glucose, requires constant vigilance and affords only imperfect glucose control, the long-term potential consequences of which are cardiovascular disease, blindness, kidney failure and damage to the peripheral nervous system.

Scientists have identified three essential steps toward developing a curative treatment: 1) a source of beta cells must be identified; 2) the immune system must be convinced not to attack those cells; and 3) the cells must be delivered into a suitable location in the body so they can exert effective control over blood glucose. However, there are still significant challenges to overcome before a practical cell-based treatment is available to the majority of type 1 diabetics.

FIGURE 1:
Schematic of healthy and diabetic pancreas. Courtesy of Amy Greenwood, PhD



Replenishing Beta Cells

A cell therapy treatment for type 1 diabetes requires the replacement of missing beta cells with new ones. Currently, there is a severe shortage of donor pancreatic tissue for transplantation, and the question of where to get more is the subject of much concern.

By studying the normal development, proliferation, and survival of beta cells in vivo (in the body), researchers attempt to learn how to manipulate these cells for therapeutic purposes. Under normal conditions, beta cells, which are clustered into structures called islets, maintain

It is known with certainty that embryonic stem cells can produce any tissue of the body, including beta cells; their directed differentiation is the subject of intense investigation.

their mass by dividing very slowly over the course of adult life. Unlike some tissues in the adult body, the pancreas appears to have limited ability to regenerate; the beta cell mass does not simply grow back if part or all of the pancreas is excised. Similarly, recent experiments have shown that the eventual size of the pancreas in adult mice is directly related to the number of pancreatic progenitors (immature cells) that exist in the embryo: the fewer the progenitor cells, the smaller the pancreas. Thus, the organ typically does not grow to accommodate body size.

Despite the limited ability of the pancreas to regenerate, there are conditions under which the beta cell mass is regulated. Although the mechanisms responsible are poorly understood, it is known that during embryonic and neonatal periods, beta cells are born and divide quite rapidly. During adulthood, the beta cell mass is often increased in individuals who are obese, insulin-resistant, or pregnant, and possibly in some cases of injury.

Unfortunately, there is no direct scientific evidence for stem cells in the adult pancreas, despite frequent assertions of their existence. The question of where and how new beta cells are generated is an important one, since the same cells that give rise to beta cells in vivo are the best ones to focus on for expansion and differentiation in vitro (in the laboratory). In addition, the identification of factors that control beta cell proliferation would clearly contribute to a cell-based therapy.

The absence of an adult pancreatic stem cell has led many researchers to investigate the potential of embryonic stem cells (ESCs). It is well known that ESCs can produce any tissue of the body including beta cells. Being able to guide their differentiation is the subject of intense investigation and a promising strategy for producing new beta cells for transplantation. Additionally, there are techniques that can be applied to ESCs that will be powerful tools in treating disease, for example therapeutic cloning to create “personalized” ESC lines to generate cells for autologous (self) grafts. ESCs can also be genetically manipulated to correct defects or add helpful attributes to cells before they are returned to the body. Since healthy beta cells in a diabetic patient are in short supply and given some of the funding restrictions on human ESCs, researchers need to overcome technical as well as financial hurdles.

Currently, there are three promising strategies to produce new beta cells for people with diabetes:

Coaxing ESCs to become beta cells in vitro: Current research is focused on directing ESCs through a series of differentiation steps that are like those that occur during normal development. Both human and mouse ESC lines have been engineered to express fluorescent molecular tags when certain molecular milestones have been reached along the pathway toward beta cell differentiation. These cell lines are being used in various screens to identify factors that promote the appropriate differentiation steps. It was also recently shown that ESCs grown under conditions that incorporate some aspects of normal pancreas development can

generate insulin-expressing cells. Although this result is encouraging, the insulin-producing cells were also observed to coexpress other hormones, indicating that they are not mature, functional beta cells.

Genes involved in beta cell development: A subset of known genes may be sufficient to confer the beta cell identity on non-beta cells through some sort of molecular intervention. Capitalizing on this may allow researchers to coax non-beta cells to assume the identity and function of beta cells. In addition, understanding genes responsible for beta cell destruction may aid in the development of methods for protecting transplanted cells.

Identify factors that increase beta cell mass: Controlling the proliferation and survival of beta cells is of great interest. Scientists would like to identify the factors responsible for the selective enhancement of beta cell mass. It may also be possible to screen either harvested (from a donor) or embryonic stem-derived beta cells in vitro for chemicals or biological agents that promote their proliferation or survival.

The most important test of cultured (grown in a laboratory) and engineered beta cells, however, is their ability to control glucose levels when transplanted in vivo. This aim will be further advanced by the development of additional immunosuppressive and surgical techniques.

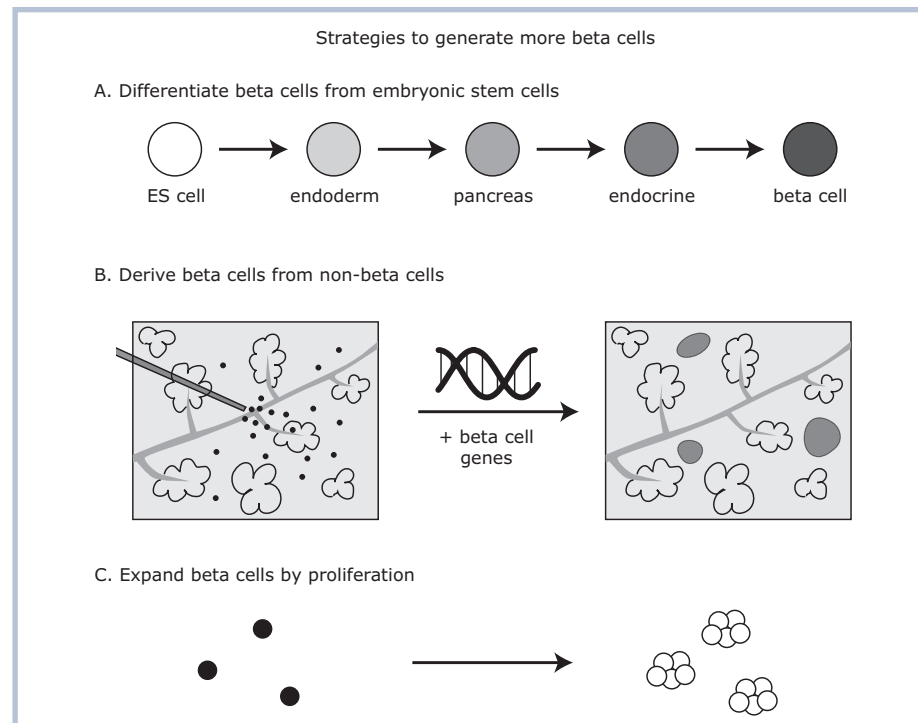


FIGURE 2: Strategies to generate more beta cells. Courtesy of Amy Greenwood, PhD

Immunological Interventions

Since type 1 diabetes is an autoimmune disease directed at beta cells, there is the need to abrogate the autoimmune attack. Additionally, most visions of a cell-based therapy for diabetes involve transplanting foreign cells, but without immune suppression, these would be quickly rejected. Due to its serious side effects, general immune suppression is not indicated for most diabetics. Thus, creating and delivering a cell-based cure to the majority of type 1 diabetics depends on the ability to selectively inhibit autogenic (self) and allogenic (non-self) immune responses.

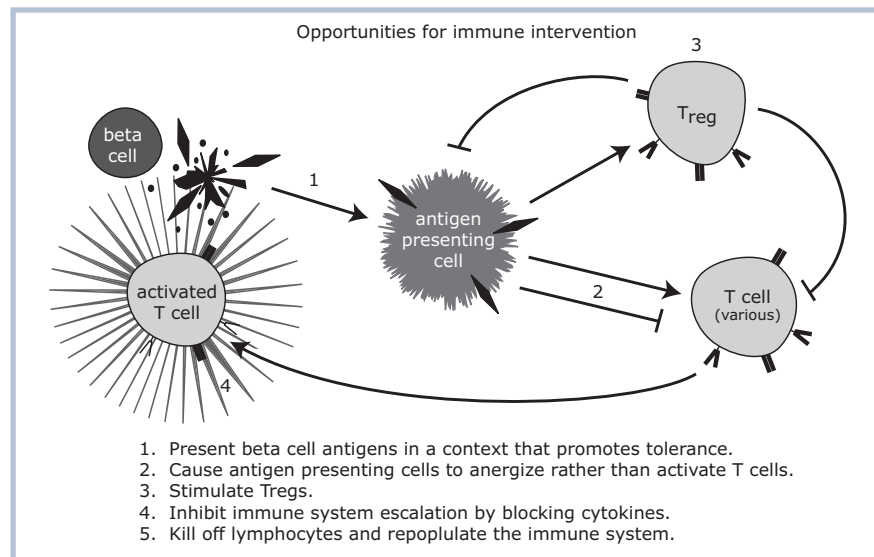
The autoimmune attack in type 1 diabetes is thought to center around a subset of lymphocytes (white blood cells) called T cells. Each T cell responds to one specific molecular fragment, called an antigen, and collectively these cells police the body for intruders. Activated T cells infiltrate pancreatic islets and start to destroy beta cells, recruiting other types of lymphocytes to the area, escalating the attack. Even after the beta cells are gone and the immune response has diminished, some lymphocytes are set aside as “memory” cells, guards prepared to respond quickly to another exposure to beta cell antigens. A similar reaction occurs in response to transplanted allogeneic beta cells, but with even greater vigor. The reason the grafted cells are attacked is no mystery; they express many foreign antigens and have the potential to activate a large number of T cell clones. Without any immune suppression, the destruction of grafted beta cells is guaranteed. It is likely that full control over autoimmunity and graft rejection will require a combination of approaches.

Alleviating the response: It may be possible to present beta cell antigens to the immune system in a manner that alleviates the response rather than induces it, such as by intravenous injection. Unfortunately, initial attempts at this method have not been successful. In a recent well-controlled clinical trial, it was shown that intravenous administration of insulin is not effective in preventing type 1 diabetes in humans. Analysis of oral administration of insulin is still ongoing.

Drug development: Significant effort has been directed toward developing biopharmaceuticals that block the signaling molecules used by activated T cells to recruit other lymphocytes. Although this approach is not entirely precise and may have side effects, it is more focused than current general immune suppressants.

Treatment: Theoretically, it is possible to eliminate lymphocytes in a type 1 diabetic and repopulate the immune system with non-reactive lymphocytes through a bone marrow transplant. A recent phase I/II clinical trial of newly diagnosed type 1 diabetics suggested that chemical immunoablation followed by a transplant of autologous hematopoietic stem cells can allow the recovery of islet function for some period of time. Given the lack of a control group, however, and the likelihood of residual islet function in the selected patients, it is possible that the entire salutary effect can be attributed to chemical destruction of the lymphocytes and will be short-lived.

FIGURE 3:
Opportunities for immune intervention. Courtesy of Amy Greenwood, PhD



The selectivity of immunologic interventions is the key to their safety. Our ability to generate antigen-specific treatments would be greatly enhanced by techniques that allowed us to expand, manipulate, and educate various types of immune cells in vitro. Given that the immune system is entirely maintained by stem cells and proliferating progenitors, this is a feasible yet distant goal. Moreover, the ability to generate cells of the immune system from ESCs would allow unprecedented faster development of potential therapies.

Transplantation of Pancreatic Tissue

New beta cells must be placed in the body in close contact with blood vessels to provide balance of glucose levels. Transplant surgeons identify patients who would benefit from a graft of pancreatic tissue, design and perform the procedure, devise immunosuppressive strategies, and monitor the outcome. Through their efforts to develop different transplant protocols, they

have shown that grafts in mice of pancreatic tissue can reduce or eliminate the need for injected insulin and ameliorate some of the complications of the diabetes, demonstrating that a cell-based cure for type 1 diabetes is feasible.

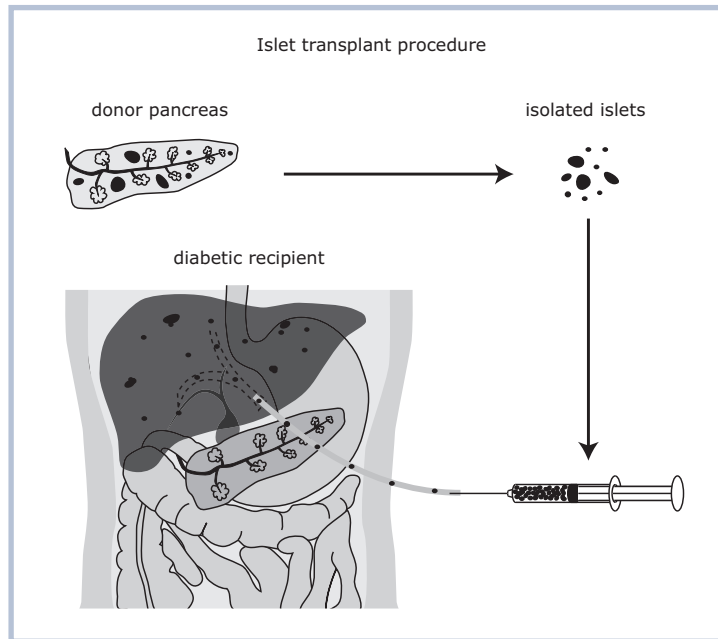
In a small study published in 2000, and a larger follow-up in 2006, a new procedure called the Edmonton protocol (University of Alberta) demonstrated a significant improvement in islet transplant success over previous procedures. Commonly used, the Edmonton protocol involves the isolation of islets from the pancreas of a cadaveric donor by special enzymatic and separation techniques, and the delivery of these islets through a catheter into the portal vein of the liver. Although similar in some respects to other islet transplantation techniques, the Edmonton protocol incorporated two important changes: the immunosuppression regimen (cocktail of drugs) was less toxic to beta cells, and significantly more islets were delivered to the patient in the form of multiple transfers. As a result, the reported rates for insulin independence at one year after islet transplant jumped from 8 percent to approximately 45 to 50 percent. However, by year two, the percentage of patients who were insulin free dropped off to less than 15 percent.

The outcome of islet transplant procedures, albeit imperfect, demonstrates that isolated clusters of cells can be used in humans to control blood sugar for a significant period of time. Thus, the whole organ is not required in the short term. However, there is variability in the initial success of the grafts, and the eventual relapse of the disease in individuals who did achieve insulin independence indicates that most of the grafts eventually fail.

Understanding why islet transplants fail is one of the main concerns of ongoing research:

- It is possible that some grafts fail because isolated islets are variable in terms of their function, or “islet potency.” Objective, rapid assays of islet function and health will be helpful in characterizing islet preparations to select those that will function best when grafted.
- It is also possible that the liver is not the optimal environment for long-term survival and function of beta cells. Other sites of delivery that have been tested in animals include the kidney capsule, thymus, spleen, omental pouch (a pocket made by the peritoneum near the intestines) and subcutaneous (underneath the skin) locations.
- Although new immunosuppressive drugs are clearly an improvement over older versions, they may still be toxic to beta cells. The development of less toxic immunosuppressants is an important focus for transplant surgery in general, and the detailed analysis of immunosuppression protocols in different patient populations is the subject of significant effort.
- Finally, despite immunosuppression, the immune system may be depleting cells in the graft because they are foreign or simply because they are beta cells.

FIGURE 4:
Islet transplant procedure.
Courtesy of Amy Greenwood, PhD



As selective methods of immunosuppression become available, scientists face the question of how and where to transplant islets. It is important to examine the roles of vascularization, function and long-term survival of the graft in order to ensure that cells behave and survive like normal beta cells when transplanted. The ability to monitor islet masses in vivo would make this goal much easier, and imaging technologies are continuing to develop. In any case, a rigorous analysis of transplant function, both in the short and long term, will need to be carried out in suitable animal models to properly assess newly developed procedures.

Embryonic Stem Cells as a Source for Beta Cells: Multiple Paths Forward

The research efforts that comprise a cell-based cure for type 1 diabetes address distinct tasks: the generation of new beta cells, the induction of selective tolerance in the immune system and the identification of the best transplant procedures for pancreatic tissue. All three areas of study are moving forward simultaneously, and advances in one area will only enhance our knowledge in another.

The ability to efficiently direct the differentiation of ES cells into either beta cells or lymphocytes would have numerous implications for immunology and implantation. Selective immune suppression would allow for better analysis of transplantation techniques and extend

the application of transplantation to a wider range of patients. Identification of transplant sites within the body or methods of encapsulating cells that support long-term survival and vascularization will allow a direct comparison between in vitro-derived beta cells and harvested pancreatic tissue, an important benchmark in evaluating new beta cells.

Although a cure for diabetes requires the consecutive completion of three difficult tasks, directing the maturation of ES cells is in progress. The commitment of financial and human resources toward the coordination of the different research arms will greatly improve the likelihood of success.

For more information about the HSCI Diabetes Program, visit the HSCI Web site at www.hsci.harvard.edu.

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