

## Diabetes and stem cells according to: Shohta Kodama and Denise L. Faustman

# Routes to regenerating islet cells: stem cells and other biological therapies for type 1 diabetes

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**Abstract:** New biological therapies for type 1 diabetes are emerging from the forefront of stem cell and islet cell biology. Basic research in animal models has uncovered a variety of mechanisms by which natural regeneration of pancreatic islet cells occurs, despite the underlying autoimmune defect. Two mechanisms – in particular,  $\beta$ -islet cell proliferation and stem cell differentiation – can be harnessed in innovative ways in order to regenerate islets lost to disease. This review provides a background on stem cells and describes a range of potential biological therapies for type 1 diabetes, including the use of adult stem cells from the spleen, an organ not previously considered a source of pancreatic stem cells. Stem cells of the spleen have been demonstrated to home to the pancreas, where they mature into fully functional islet cells responsible for restoring normoglycemia. If the underlying autoimmune defect can be eradicated, stem cells of the spleen, as well as related strategies, can be used in order to regrow islets destroyed by type 1 diabetes.

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## Introduction

The regeneration of pancreatic  $\beta$ -islet cells for type 1 diabetes is a longstanding research goal. New ways to regenerate (i.e., replenish)  $\beta$ -islet cells destroyed by autoimmune disease have been generated by means of the explosion of interest in stem cells. While most research attention has been on transplanting stem cells from exogenous sources, many other regenerative therapies are emerging to harness the body's endogenous sources – a strategy with inherently lower risks of immune rejection.

New therapeutic strategies arise from growing understanding of the diverse mechanisms of regeneration, some of which have been found to persist throughout life. For decades, biologists believed that humans had only limited capacity for regeneration. That paradigm has changed in the wake of finding at least four mechanisms of regeneration, including replication of fully differentiated cells (such as  $\beta$ -islets), recruitment

and differentiation of stem cells, cell fusion, and, more rarely, transdifferentiation (of one stem cell type to another) (Table 1). These regenerative mechanisms likely co-exist in the same organism. The host, the underlying disease, and the administered treatments may dictate what mechanisms are tapped.

For type 1 diabetes, at least two of these mechanisms –  $\beta$ -cell replication and stem cell differentiation – might be marshaled in previously unforeseen ways for developing new therapies. These approaches are possible, because the underlying defect is autoimmune and does not affect the regenerative capacity of islets or their precursors (1, 3–5). If the underlying autoimmune attack on  $\beta$ -cells can be eliminated through progress in research, then the regenerative capacity could be cultivated for therapeutic purposes.

This study covers a range of potential therapeutic strategies, from promoting mature  $\beta$ -islet cells to proliferate to using stem cells in unusual ways. One

Table 1. Types of regeneration

Cell division	Self-duplication by mitosis
Stem cell differentiation	Maturation of a stem cell into a specialized cell (e.g., $\beta$ -islet cell). A stem cell has two features: the ability to divide over the long term and to differentiate into one or more specialized cell types.
Transdifferentiation	Conversion of a stem cell or a differentiated cell of one lineage to a cell with a different lineage (e.g., a neuron stem cell becomes an endothelial cell)
Fusion	Fusion of two cells into one cell in order to survive

involves harvesting a new population of islet stem cells found in the spleen (2). Other strategies are to promote the regeneration of endogenous islet stem cells or to encourage spontaneous regeneration without the need for formal treatment. For any of these potential therapies to be successful, the underlying defect in type 1 diabetes – an autoimmune attack on  $\beta$ -cells – must be eliminated. Removal of this defect is arguably a more difficult challenge.

### Regeneration by islet cell replication

In normal animals without diabetes,  $\beta$ -islet cells are most likely replaced, throughout life, by the replication of mature  $\beta$ -islets. While recruitment of stem cells to differentiate into  $\beta$ -cells might supplement this natural turnover, lineage tracing found replication to be the main mechanism of regeneration in normal mice without natural disease (6). In humans with type 1 diabetes, case reports have established that the regeneration of islet cells still occurs years after the onset of the disease (3–5). Although the mechanisms of regeneration have not been formally studied in humans, acceleration of  $\beta$ -cell replication could be a mechanism of islet cell regrowth in type 1 diabetes. Therapies could be designed in order to stimulate  $\beta$ -islet cell regeneration, but there has not been much research devoted to this concept. It would be ideal to find a mitogen that is specific to  $\beta$ -islet cells and that also does not induce tumors in the host.

### Regeneration by stem cell differentiation

Stem cells hold much promise for regenerating or regrowing any tissue or organ, given that they possess two essential features: the capacity to divide over the long term (a property known as self-renewal) and the capacity to give rise to a range of specialized cell types. Stem cells found in the human pancreas, e.g., can divide for lengthy periods and differentiate into  $\beta$ -islet cells that secrete insulin (7, 8). Others have proposed that islet cells emerge from duct cells (9). Stem cell differentiation *in vivo* is, generally, controlled by

exposure to growth factors and other signals in the immediate extracellular milieu surrounding these cells.

Stem cells can be found in tissues of any age – embryos (embryonic stem (ES) cells), fetuses, and adult tissues. ES cells are the most controversial, because some groups believe that the destruction of an embryo in order to obtain the cells is equivalent to murder. Adult stem cells are less ethically controversial and, to escape immune rejection, can be harvested from the same person. The qualifier ‘adult’ falsely implies that the stem cells are found only in adults. Adult stem cells, in fact, are undifferentiated cells that reside in differentiated tissues found in fetuses, infants, children, and adults. Adult simply refers to the highly differentiated tissue that houses the stem cells.

Adult stem cells are known to exist in a multitude of tissues in animals and in humans (Table 2). Most adult stem cells are restricted to becoming specialized cells of the organ or tissue in which they reside. The restricted lineage capacity of adult stem cells is the major difference between them and ES cells.

ES cells can differentiate *in vitro* and *in vivo* to form a wide range of specialized cell types. Taken from the embryo at the blastocyst stage, ES cells are capable of creating all cell types in an embryo (i.e., pluripotent), as well as a whole animal. Their versatility is an asset over adult stem cells, but also a challenge. While ES cells can become insulin-secreting cells in culture, e.g., the cells are not as stable as adult stem cells. ES cells studied *in vitro* and *in vivo* can differentiate into tumor cells. Similarly, the rapid proliferation rate of ES cells, which is also greater than that of adult cells, carries greater risk of forming tumors *in vivo* (10, 11).

Adult stem cells vary in their capacity to form new cells and tissues for the purpose of regeneration. The mechanisms employed by various pockets of stem cells in order to promote tissue repair and normal tissue turnover are diverse. Those mechanisms may be intricately linked to the host condition. One mechanism might be for maintaining homeostasis. Another might be tapped for repair after injury, and yet another for repair with disease.

### Regeneration by stem cell transdifferentiation

The possibility that adult bone marrow stem cells have the capacity to transdifferentiate – that is, to jump tissue lineage boundaries – raised great expectations. Researchers hoped, e.g., to convert hematopoietic stem cells (HSC) to non-hematopoietic cells, such as the heart, the brain, the gut, the liver, and the muscle. Because bone marrow or peripheral blood offers an accessible and plentiful supply of HSC, they were seen as ideal sources from which to harvest HSC for treating disease. Several studies in animal models claimed that HSC could differentiate into the heart, lung, brain, and pancreatic islets (12–14). The evidence spurred human

Table 2. Adult human stem cells and their primary direction of differentiation

Cell type	Tissue-specific location	Cells or tissues produced	References
Hematopoietic stem cells (HSC)	Bone marrow, peripheral blood	Bone marrow and blood lymphohematopoietic cells	(29)
Mesenchymal stem cells	Bone marrow, peripheral blood	Bone, cartilage, adipose tissue, muscle, stroma, neural cells	(30)
Splenic stem cells	Spleen	Pancreatic islets, pancreatic ducts, etc.	(2)
Neural stem cells	Ependymal cells, astrocytes (subventricular zone) of the central nervous system	Neurons, astrocytes, oligodendrocytes	(31, 32)
Hepatic stem cells	In or near the terminal bile ductules (canals of Hering)	Oval cells for hepatocytes and ducts	(33)
Pancreatic stem cells	Intra-islet, nestin-positive cells, oval cells, duct cells	$\beta$ -cells	(21, 34)
Skeletal-muscle stem cells or satellite cells	Muscle fibers	Skeletal muscle fibers	(35, 36)
Stem cells of the skin (keratinocytes)	Basal layer of the epidermis, bulge zone of the hair follicles	Epidermis, hair follicles	(37, 38)
Epithelial stem cells of the lung	Secretion by tracheal cells, bronchiolar Clara cells, alveolar type-II pneumocyte	Mucous and ciliated cells, pneumocytes	(39)
Stem cells of the intestinal epithelium	Epithelial cells located around the base of the crypts	Paneth's cells, brush-border enterocytes, mucus-secreting goblet cells, enteroendocrine cells of the villi	(40, 41)

clinical trials of bone marrow transplants for myocardial infarction, ischemic heart disease, limb ischemia, retinopathy, renal disease, and neurodegenerative disease.

But major questions have been raised about the proportion of cells undergoing transdifferentiation and whether it was occurring at all. Studies found that HSC transdifferentiation was infrequent; most studies revealed that less than 2% of the differentiated target cell reappeared as part of the functional organ; other research groups argue that HSC do not differentiate into any parenchymal cells (15). Other studies found that new cells, which appeared not to be transdifferentiated, could be accounted for by other forms of regeneration. In some cases, the HSC had fused with the host cell, rather than transdifferentiated (16, 17). In others, the HSC transplants contained endothelial progenitor cells that had stimulated regeneration by endogenous cell division, such as by  $\beta$ -cells of the pancreas (18).

The debate over transdifferentiation led to the development of rigorous criteria for establishing its existence and efficacy (19). These criteria also are valuable for evaluating stem cell differentiation, i.e., whether purported stem cells – embryonic or adult – are maturing into functional cells in the host. The criteria are robustness, durability, and functionality. Taking  $\beta$ -islet stem cells as an example, research would need to show that a relatively high fraction of transplanted stem cells give rise to  $\beta$ -islet cells instead of other cell types (robustness), they keep producing insulin for long periods (durability), and they induce normoglycemia (functionality). Another major concern with stem cells, particu-

larly ES cells, is their safety. Cultured ES cells have been found to display chromosomal ‘instability’, a sign of malignant transformation (20). By contrast, adult stem cells are, generally, considered more stable, because, by definition, they are more restricted in terms of the specialized cell types they can become.

To meet the criteria set by Anderson and colleagues – robustness, durability, and functionality (19) – requires sensitive methods of detection. In the past, researchers claiming stem cell regrowth of an organ merely quantified the number of differentiated cells in tissue cross sections. That method, however, does not track stem cells’ fate or functionality in host tissues. Instead of transdifferentiating, the stem cells may only facilitate healing by the host, e.g., by their release of growth factors to stimulate host cell replication. In order to distinguish between the two, researchers are now employing tracking methods, such as green fluorescent protein labeling and/or sex chromosome-tracking methods. These or other lineage-tracking methods set the highest standard for proving regeneration from differentiating or transdifferentiating stem cells.

### A range of potential stem cell therapies for type 1 diabetes

Researchers are studying a broad range of stem cell therapies with the potential to form insulin-secreting cells of the pancreas. The most obvious is an adult stem cell from the pancreas (21, 22). But numerous other stem cell types, ranging between ES cells and adult HSC, are being explored (Table 3). and more

Table 3. Proposed  $\beta$ -cell sources for therapy

Cell types	Tissue-specific location	Cells or tissues produced	References
Hematopoietic stem cells (HSC)	Bone marrow, peripheral blood	May directly differentiate or may promote self-regeneration	(18, 29, 42, 43)
Splenic stem cells	Splenic capsule	Differentiates into $\beta$ -cells and duct cells of the pancreas	(2)
Nestin stem cell	Pancreas	Pancreatic insulin-secreting cells	(44)
Ductal stem cell	Ductal cells located within the pancreas	Ducts may bud new islets in the pancreas	(45)
$\beta$ -cell division	Existing $\beta$ -cells in the pancreas	$\beta$ -cell to $\beta$ -cell replication	(6, 46)
Pancreatic stem cells	Pancreas	$\beta$ -cells, brain, and muscle cells	(22)
Oval cells	Liver	Transdifferentiation of oval cells into insulin-producing cells in culture	(47)

sources are likely to be uncovered, considering the importance of pursuing a broad-based approach. It may turn out that the choice of sources varies according to disease course and severity, genetic factors, past treatments, or co-occurring conditions.

### The spleen as a potential source of stem cells

Medicine has long viewed the spleen as a disposable organ in injury and disease. Yet, while searching for an immunotherapy to treat the underlying autoimmune defect in type 1 diabetes, our laboratory unexpectedly discovered in mice a heretofore uncharacterized stem cell population in the spleen. We first showed that splenocytes taken from donor mice could, when infused with other immune therapies, contribute to reversal of the autoimmune defect in non-obese diabetic (NOD) mice, an animal model of autoimmune diabetes (1). We also showed in the same study that donor spleen cells resulted in very rapid islet regeneration. Our study and that of others (18) did not observe direct lineage development of bone marrow-origin cells in the islets. In a subsequent study, we showed with two lineage-tracking techniques that donor spleen cells had, after intravenous transfer, homed to the host's pancreas where they differentiated into  $\beta$ -islet cells (2). Not all spleen cells, however, were capable of forming new islets in diabetic mice; only non-lymphoid spleen cells (CD45<sup>-</sup> cells) could directly contribute to the newly formed islets. Our unpublished research further suggests that donor cells are not destined to differentiate into specialized spleen cells, so their maturation to pancreatic islet cells would not have been by transdifferentiation, but rather by stem cell differentiation. Because this population of newly identified spleen stem cells restored normoglycemia over a long period of time, and sufficient numbers were available from several donors without the need for expansion in tissue culture, our findings met the three criteria for stem cell efficacy: robustness, durability, and functionality (19). Our study reveals that stem cells can contribute to effectively treat a disease and can exhibit long-term functional durability.

In order to induce pancreatic islet regrowth, we selected donor cells from normal mice as a means of distinguishing them from host cells and tracking their fate. Yet, we also found that the host's islets could regenerate spontaneously, although more slowly, without any cellular therapy, as long as we eliminated the underlying autoimmune disease. In other words, spontaneous regeneration was sufficient by itself to allow for islet regeneration.

Our experience with donor splenocytes, as well as other lines of evidence, supports our hypothesis that the spleen may be a natural source of islet stem cells that is tapped by the body in specific conditions, such as diabetes and or pancreatic disease. For humans with chronic pancreatitis, e.g., the removal of the left pancreas (hemi-pancreatectomy (Px)) also involves removing the attached spleen because of shared vasculature. Only patients with left hemi-Px, compared to right hemi-Px that does not involve removing the spleen, succumb years later to insulin-dependent diabetes (23, 24). Similarly, children with severe thalasemias, who had their spleen removed, eventually develop insulin-dependent diabetes (25, 26). A possible explanation for these and related findings (27) is that the spleen is a natural harbor for islet stem cells, and its removal deprives ill people of a vital source for regenerating islets, especially in disease states of the pancreas.

It is yet to be established whether the spleen in normal people without disease contributes to the  $\beta$ -cell populations for islet regeneration. Indeed, without disease, the murine evidence suggests that  $\beta$ -cell turnover is sufficient for islet maintenance and normal blood sugar levels (6). However, mutant mice have been created with ablation of critical genes controlling exocrine pancreas development – e.g.,  $p48^{-/-}$  mice. Islet development occurs unhampered, and the mice are born normoglycemic; the islets form in the spleen in lieu of the missing pancreas (28).

It should be stressed that we do not recommend spleen cell transplants for treating type 1 diabetes. Until more is known about cellular therapies, the safest way to restore normoglycemia is first by removing the autoimmune disease and then by allowing islet cells to

Table 4. Stimulation of  $\beta$ -islet cell regeneration with compounds: animal and human studies

Compound or agent	Purposed mechanisms of action	Current research
Islet neogenesis gene-associated protein (INGAP) Interferon- $\gamma$ (INF- $\gamma$ )	Growth factor that may stimulate $\beta$ -cell growth Overexpression of INF- $\gamma$ in mice induces epithelial cell proliferation and islet regrowth	In clinical trials for type 1 and type 2 diabetes Animal studies
Glucagon-like peptide-1 (GLP-1); exendin-4	Stimulate the release of insulin from existing $\beta$ -cells; perhaps stimulates $\beta$ -cell division	Human clinical trials
Hepatocyte growth factor (HGF) Gastrin and epidermal growth factor (EGF)	Observed tissue culture expansion Observed normal control animal expansion of islet cell	Human islets in culture Animals with chemically induced diabetes

regenerate spontaneously from either minor pockets of existing islets in the pancreas or other normal adult stem cell populations, as discussed below.

### Regeneration by the promotion of endogenous or spontaneous regeneration

Studies on animals and humans now support an entirely different therapeutic approach to islet regeneration. If there are a sufficient number of endogenous islet precursors (i.e., stem cells or more differentiated cells), therapy could (i) stimulate their cell division and differentiation or (ii) allow the process to occur spontaneously, without formal treatment, assuming again that the underlying immune defect is eliminated. Each approach applies to stem cells in the pancreas or the spleen.

Certain growth factors and other substances have been found to stimulate  $\beta$ -cell reappearance in animals (Table 4). Indeed, at least five compounds are candidates for stimulating islet regeneration from endogenous sources in the mouse. For instance, the islet neogenesis-associated protein gene is a candidate, as well as glucagon-like peptide-1 (also known as exendin-4). Animal models and tissue culture experiments suggest that more candidates could be identified. Most of these candidates are growth factors, such as hepatocyte growth factor (HGF), gastrin and epidermal growth factors (EGF), or interferon- $\gamma$  (INF- $\gamma$ ) itself.

This strategy of accelerating endogenous regeneration assumes that the disease pathophysiology in type 1 diabetes only affects the immune system and does not alter islet cell regenerative capacity, whether stem cell differentiation or  $\beta$ -cell division. Indeed, evidence from the NOD mouse and human type 1 diabetes indicates the ongoing islet cell proliferation despite disease. Whether stimulating islet regrowth with stem cell injections or growth factors could tip the balance sufficient to restore normoglycemia has not yet been studied. It is also unknown whether these islet stimulatory therapies could be harmful. One possibility is that such therapies, by inducing rapid recruitment of stem cells, could consume a finite supply of stem cells, or could exacer-

bate the underlying immune attack, given evidence of immune system stimulation by a fresh supply of islet targets. Another safety concern is that islet stem cells, if rapidly induced, could become tumorigenic.

The strategy of spontaneous regeneration does not require any active treatment except tight blood sugar control, a long-standing clinical management tool to prevent diabetes complications (1). In murine animal models, under diverse experimental conditions, autoimmune disease elimination can result in a very brisk return of islets in the pancreas without the need for transplantation. The most vigorous regeneration occurs with tight blood sugar control (3). Islets gradually appear in the pancreas over the course of 120 d. The drawback of this strategy is that the natural time course for regeneration may be too long or not vigorous enough for return of normoglycemia.

### Conclusions

This review has discussed several potential biological therapies for type 1 diabetes, with particular focus on pancreatic  $\beta$ -cells and stem cells in the pancreas or the spleen. Diabetes therapy, in the future, could involve the regeneration of insulin-secreting cells by  $\beta$ -cell replication or stem cell differentiation. Neither approach to replenishing islet cells can be curative, unless the underlying autoimmune defect is eliminated. Although type 1 diabetes has been the focus of this review, several of the strategies discussed in this study might have potential therapeutic value for type 2 diabetes where recurrent islet death from immune system attack is not a problem, as long as the disease process does not alter regenerative capacity.

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