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Vivian M. Lee, Markus Stoffel

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Commentary

Diabetes, a leading cause of morbidity and mortality in industrialized countries, is caused by an absolute insulin deficiency due to the destruction of insulin secreting pancreatic β cells (type 1 diabetes) or by a relative insulin deficiency due to decreased insulin sensitivity, usually observed in overweight individuals (type 2 diabetes). In both types of the disease, an inadequate mass of functional β cells is the major determinant for the onset of hyperglycemia and the development of overt diabetes. Maintenance of pancreatic β cell mass results from a dynamic balance of neogenesis, proliferation, and apoptosis (1). These processes are adaptive since β cells can proliferate physiologically in postnatal life (during growth or pregnancy), in response to injury, or in disease states such as obesity or other genetic forms of insulin resistance (2, 3). Therefore, the identification of pancreatic precursor (stem) cells and the mechanisms controlling their proliferation and differentiation are of central importance for developing novel approaches to treat diabetes. Pancreatic islet development from gut endoderm Endocrine and exocrine cells of the pancreas are derived from a common set of epithelial cells from early gut endoderm (4). Although insulin expression can first be detected shortly after pancreatic bud formation, these cells are not believed to be the precursor of differentiated islet cells (5). Differentiated β cells first appear around embryonic day […]

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Bone marrow: An extra-pancreatic hideout for the elusive pancreatic stem cell?

Vivian M. Lee and Markus Stoffel

Laboratory of Metabolic Diseases, The Rockefeller University, New York, New York, USA

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Diabetes, a leading cause of morbidity and mortality in industrialized countries, is caused by an absolute insulin deficiency due to the destruction of insulin secreting pancreatic β cells (type 1 diabetes) or by a relative insulin deficiency due to decreased insulin sensitivity, usually observed in overweight individuals (type 2 diabetes). In both types of the disease, an inadequate mass of functional β cells is the major determinant for the onset of hyperglycemia and the development of overt diabetes.

Maintenance of pancreatic β cell mass results from a dynamic balance of neogenesis, proliferation, and apoptosis (1). These processes are adaptive since β cells can proliferate physiologically in postnatal life (during growth or pregnancy), in response to injury, or in disease states such as obesity or other genetic forms of insulin resistance (2, 3). Therefore, the identification of pancreatic precursor (stem) cells and the mechanisms controlling their proliferation and differentiation are of central importance for developing novel approaches to treat diabetes.

Address correspondence to: Markus Stoffel, The Rockefeller University, Laboratory of Metabolic Diseases, 1230 York Avenue, Box 292, New York, New York 10021, USA. Phone: (212) 327-8797; Fax: (212) 327-7997; E-mail: stoffel@mail.rockefeller.edu.

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Nonstandard abbreviations used: embryonic day (E); enhanced green fluorescent protein (EGFP); glucose transporter 2 (Glut-2); hematopoietic stem cell (HSC); multipotential adult progenitor cell (MAPC); tyrosine kinase Kit (CD117).

Pancreatic islet development from gut endoderm

Endocrine and exocrine cells of the pancreas are derived from a common set of epithelial cells from early gut endoderm (4). Although insulin expression can first be detected shortly after pancreatic bud formation, these cells are not believed to be the precursor of differentiated islet cells (5). Differentiated β cells first appear around embryonic day 13 (E13) at the onset of the secondary transition, a phase of pancreatic organogenesis during which endocrine cells detach from the exocrine matrix, increase in number, and reorganize to form mature islets. Morphologically distinct cell clusters that exhibit the typical architecture of mature islets containing all endocrine cell types are first detected at about E17.5. During the perinatal period, β cells of the islet undergo final differentiation, as evidenced by their ability to become glucose responsive in the first week of life.

Islet growth continues after birth, resulting from a combination of both an increase in cell size (hypertrophy), as well as cell number (6, 7). In addition, throughout much of life, small islets continue to develop from pancreatic ducts through neogenesis and proliferation. Islet mass turnover in rodents is slow and is believed to derive from two sources: replicating β cells in pancreatic islets and neogenesis from pancreatic ducts (8). The capacity of β cells to replicate is certainly important in the postnatal period, but may be more limited at later stages in life. Neogenesis of islets from pancreatic ducts, a two-step process that involves the expansion of duct epithelium and subsequent differentiation into mature islet cells, constitutes a second neogenic pathway that has been studied extensively in rodent models of pancreatic regeneration. This process is also believed to contribute to increased islet mass in mouse models of extreme insulin resistance where islets are often found in the vicinity of proliferating ducts. It should be noted that the concept of islet regeneration from duct epithelium has recently been challenged by lineage tracing studies that show an early separation of adult duct progenitors from endocrine cell lineages (9). Thus, the role of ductal epithelium in islet regeneration needs further investigation.

Bone marrow: an extra-endodermal source of islet β cells

In this issue of the *JCI*, Ianus and colleagues (10) report an extra-pancreatic source of pancreatic β cells that may play a role in β cell turnover and possibly the adaptation of islet mass in response to physiological and environmental stimuli. In this elegant study, bone marrow cells that selectively express the enhanced green fluorescent protein (EGFP) if the insulin gene is actively transcribed, were transplanted into lethally irradiated recipient mice and gave rise to

EGFP-positive insulin-producing cells in pancreatic islets. These bone marrow–derived cells expressed islet-enriched genes such as the glucose transporter 2 (*Glut-2*) and several transcription factors that are markers of β cell differentiation. Moreover, these cells seemed to be functional since glucose and incretins stimulated insulin secretion. Through a genetic (Cre-loxP) approach, the authors ruled out cell fusion as the mechanism for EGFP-positive cells with islet-like characteristics.

The demonstration that bone marrow cells can contribute to pancreatic islet mass raises a number of interesting questions with regard to the nature of these stem cells and their differentiated progeny, such as: the mechanism of differentiation, the kinetics of bone marrow–derived β cell homing and turnover, physiological characteristics, and their role in islet mass expansion (β cell compensation) in disease states such as insulin resistance.

Origin of the pancreatic progenitors found in bone marrow

There are two possible ontogenetic sources of the pancreatic precursors derived from bone marrow. One possibility is that bone marrow stem cells are nonendodermal, with no immediate relationship to putative pancreatic stem cells that are resident in tissues of endodermal origin or developmental neuroendocrine stem cells derived from the endoderm. Alternatively, stem cells in bone marrow may be derived from sites of endodermal origin. For example, it is possible that during development endocrine precursors enter the circulation and reside in an extra-endodermal organ such as the bone marrow. Regardless of their germ layer of origin, these cells would represent multipotent cells that, mediated by circulating signals, can be recruited to neuroendocrine compartments of the pancreas. It is unclear as to whether these precursors may first migrate to intermediate locations, such as the pancreatic duct epithelium, before final differentiation in the pancreatic islets. Thus, it is possible that these bone marrow–derived progenitors may be the same as other progenitor cells previously postulated. Once homing of these cells to pancreatic islets has occurred, local cell-cell interaction as well as paracrine factors may initiate differentiation. The experimental design of the study by Ianus et al. (10) only allowed them to test if bone marrow cells could differentiate into insulin-transcribing β cells. Future studies will have to shed light on whether bone marrow stem cells give rise to other islet cells, such as somatostatin-, glucagon-, and PP-expressing cells.

Candidate pancreatic progenitors in bone marrow

The subpopulation of bone marrow cells that can give rise to insulin-positive islet cells is unknown. One candidate is the bone marrow hematopoietic stem cell (HSC) that has been shown to differentiate into hepatocytes (11, 12). However, a recent single cell bone marrow transplantation study using EGFP-marked HSCs indicates that these cells robustly reconstitute peripheral blood leukocytes in lethally irradiated recipient animals, but do not appreciatively contribute to nonhematopoietic tissues including endodermally–derived organs such as liver and gut (13). Although pancreatic tissue was not specifically investigated in this study, the data indicate that transdifferentiation of circulating HSCs is an extremely rare event.

A more likely candidate may be a recently described multipotential adult progenitor cell (MAPC) derived from adult bone marrow (14). These cells are more restricted in their self-renewal capability but exhibit remarkable plasticity with the ability to differentiate into cells with mesodermal, neuroectodermal and endodermal characteristics in vitro. Furthermore, upon transplantation, MAPCs can differentiate into epithelium of the liver, lung, and gut.

Of interest in this regard is the receptor tyrosine kinase Kit (CD117) that is routinely used as a stem cell marker in the hematopoietic system. Kit expression is also detectable in epithelium of the developing pancreas. These cells are negative for both insulin and glucagon; however, they express Pdx-1 (pancreas/duodenum homeobox gene 1) and Pax-6 (paired box homeotic gene 6) indicating that they may represent endocrine precursor cells (15). It could be postulated that these cells contribute to the Kit-positive stem cell pool of the bone marrow. Interestingly, about 10–20% of insulin-positive cells in the adult islet also express Kit, perhaps representing the contribution of bone marrow–derived cells to islet mass.

Role of bone marrow in the maintenance of islet mass

Another interesting question that arises from the present study involves the kinetics of bone marrow–derived engraftment of pancreatic islets. Approximately 2–3% of bone marrow–derived EGFP-positive cells were detected by cell sorting of recipient pancreatic islet cells four to six weeks after bone marrow transplantation, with bone marrow engraftment efficiency estimated at 70–90%. Quantitative measurements estimate β cell turnover in adult rat islets to be as high as 3% per day (6). If β cell turnover rates in rats and mice are similar, then the contribution of bone marrow–derived β cells may be quite small. However, it should be noted that the present study was not designed to address this question. Similar studies allowing multiple engraftment intervals will allow more precise estimations of bone marrow cell contribution to total islet mass over time. Furthermore, engraftment kinetics may be useful in determining differences in the ability to generate pancreatic insulin-producing cells from the bone marrow of mice from different age groups, as well as mice with metabolic disturbances that lead to increased (e.g., insulin resistance) or decreased (e.g., advanced age) islet mass. It is well established that mice of different genetic backgrounds differ in their ability to increase islet mass in response to insulin resistance. Thus, genetic factors may also significantly influence the capacity for bone marrow cells to engraft the pancreas.

The data presented by Ianus et al. (10) suggest that pancreatic islet cells derived from bone marrow may be functional: they expressed insulin and β cell specific markers, and responded to glucose and other insulin secretagogues. It is known that there is functional heterogeneity among islet β cells, including differences in insulin granules, insulin content, glucose responsiveness, and gene expression (15–18). Future studies will need to establish whether bone marrow–derived islet cells also exhibit such heterogeneity, or contribute to a specific subpopulation.

β cell replacement therapy by islet transplantation has recently been shown to restore normoglycemia in type 1 diabetics (19). However, a limited supply of human islet tissue prevents this therapy from being used to

treat the thousands of patients with type 1 diabetes. The use of bone marrow as a source of pancreatic β cell progenitors has the potential for ex vivo expansion, differentiation, and autologous transplantation. Thus, immunosuppression to prevent rejection could be avoided. Identifying the subpopulation in the bone marrow that gives rise to functional insulin-secreting cells, the mechanism of islet engraftment, as well as the environmental signals that trigger differentiation will be essential for exploiting these cells for the treatment of type 1 and possibly some forms of type 2 diabetes.

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The challenge of molecular medicine: complexity versus Occam's razor

Eric A. Sobie,[1,2] Silvia Guatimosim,[1] Long-Sheng Song,[1] and W.J. Lederer[1]

[1]Medical Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore, Maryland, USA
[2]Nora Eccles Harrison Cardiovascular Research and Training Institute, University of Utah, Salt Lake City, Utah, USA

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Everything should be made as simple as possible, but not simpler.

—Albert Einstein's comment on Occam's Razor

The goal of molecular medicine is to find treatments for human diseases by the clever and effective application of the tools of molecular and cell biology. To do this, an animal model (or a set of animal models) of the disease is devised, investigated, and characterized. Novel therapies are conceived and tested on the animal model(s) until a rescue from the pathology is achieved. The rescue strategy is then developed for human trial.

Cellular cybernetics

In the spirit of the recent elucidation of the human genome and the current scientific epoch of bioinformatics, a brute-force therapeutic strategy would theoretically be a perfect remedy for any disease. The essential idea is both comprehensive and unsubtle; the strategy only requires that the therapy fix what was broken. While that sounds simple and possible in this era of rapidly advancing medicine, proteomics, genomics, and targeted pharmacology, it is not. The problem is that the level of complexity of most diseases is great (see Figure 1) and our present knowledge of physiology and pathology is inadequate to undertake such comprehensive repair. Indeed, even nominally simple diseases appear to rapidly develop complexities beyond our current grasp. In the absence of such complete knowledge, current endeavors in molecular medicine are guided by Occam's razor, or the idea that the simplest therapy for the animal model is likely to be effective in treating human disease. This approach is very much in the engineering tradition of fixing what the available tools permit and evaluating alternatives based on empirical rather than theoretical considerations. While this is a reasonable approach, the complexities of many

Address correspondence to: W. J. Lederer, Medical Biotechnology Center, University of Maryland Biotechnology Institute, 725 W. Lombard Street, Baltimore, Maryland 21201, USA. Phone: (410) 706-8181; Fax: (410) 510-1545; E-mail: lederer@umbi.umd.edu.

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Nonstandard abbreviations used: muscle LIM protein (MLP); phospholamban (PLN); cardiac sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a); β-adrenergic receptor kinase–1 (βARK1); sarcoplasmic reticulum (SR); mutant myosin binding protein C (MyBP-C_{MUT}).