Islet replacement via the Edmonton Protocol offers proof of concept that transient insulin independence can be achieved in patients with diabetes (1–4). However, the need for lifelong immunosuppression, an extreme shortage of human islets, along with graft failure due to rejection and continued autoimmunity, limits the widespread application of this approach. Although recent progress has been made in the derivation of “β-like” cells from human pluripotent stem cell sources (embryonic stem cells or induced pluripotent stem cells) (5–10), inducing the replication of surviving β-cells in situ (11, 12) and/or stimulating new islet formation from progenitor cells within the pancreas (islet neogenesis) (13–16) represents the most direct way to increase β-cell mass.

Studies in both rodents and humans suggest endogenous islet repair mechanisms may remain intact during diabetes and can be activated by cell- or pharmacologically-mediated stimuli. Evidence of ongoing β-cell regeneration has been observed in type 1 diabetes (T1D) patients at early onset (17) or many years after diagnosis (18). Indeed, recent analysis of T1D patients with disease duration of more than 50 years (medalists) has uncovered sustained c-peptide and proliferating β-cells within the pancreas in the face of ongoing autoimmunity (19). Moreover, the increase in β-cell mass in response to pregnancy suggests the human endocrine pancreas has the capacity to regenerate if we can understand how to “tip the balance” in favor of islet regeneration vs destruction during diabetes. Interestingly, increased β-cell replication was first suggested as the mechanism for increased β-cell mass during pregnancy, but a recent autopsy study of human pancreases during pregnancy showed an increased proportion of small islets and increased number of insulin+ cells in the ducts but no change in β-cell replication or apoptosis frequency (20). Nonetheless, novel strategies to optimize islet regeneration, either by stimulating β-cell replication or by harnessing endogenous islet neogenic processes within the diabetic pancreas, remain under intense investigation for the treatment of diabetes.

In initial publications documenting endogenous islet regeneration by stem cells, transplantation of bone marrow-derived c-kit+ cells reduced hyperglycemia in mice with streptozotocin (STZ)-induced β-cell deletion (21). Importantly, transplanted stem cells did not acquire insulin expression as previously thought. In contrast, donor cells surrounded regenerating islets or engrafted in ductal regions and stimulated proliferation within recipient islets via undetermined paracrine activities (21). Subsequently, other preclinical and early clinical reports have shown that both human hematopoietic (22–26) or multipotent stromal cell (25–28), also known as mesenchymal stem cell (MSC) lineages, can enhance islet regeneration. Furthermore, islet regeneration and immune protection may be achieved by coinfusion of murine hematopoietic cells with MSC in autoimmune non-obese diabetic mice (29). Although this concept termed “stem cell-stimulated islet regeneration” has emerged as a central process for pancreas repair, either by stimulating β-cell proliferation (11, 24–26) or by initiating new islet formation from ductal or islet-derived precursors (14–16, 25, 26), the molecular signaling mechanisms by which distinct stem and progenitor cell types mediate islet recovery are not well understood. More importantly, recent controversy of the role of betatrophin in islet regeneration (30, 31) has underscored
the importance of prospective identification and functional validation of secreted effectors that stimulate islet regeneration before translation of this concept into a clinically relevant treatment for diabetes.

Deciphering Intrinsic Signals That Stimulate Islet Regeneration

In this issue of Endocrinology, Smid et al (32) performed a series of gene and protein expression experiments to identify the secreted factor periostin, a protein implicated in the induction of epithelial-mesenchymal transformation, as a novel mediator of pancreatic endocrine regeneration after partial (70%) pancreatectomy. Notably, periostin contains a vitamin K-dependent carboxylation/γ-carboxyglutamic (Gla) domain responsible for the high-affinity binding of calcium ions. As such, periostin is efficiently integrated into the surrounding extracellular matrix (ECM) and functions as a ligand for α-V/β-3 and α-V/β-5 integrin receptors to support adhesion, survival, and migration of epithelial cells (for review, see Ref. 33). This group has previously used a similar strategy to also identify the role of Wnt7a in myogenic stem cell-mediated muscle repair (34–38), demonstrating that studying intrinsic regeneration and repair mechanisms in adult tissues can be used to identify elusive matrix-bound or soluble factors that serve as molecular mediators of regenerative processes.

Periostin mRNA was more than 80-fold up-regulated within the tip of the pancreas remnant compared with the intact tail fragment tissue 3 days after partial pancreatectomy, and periostin protein was highly expressed by resident mesenchymal stromal cells termed pancreatic stellate cells (PSCs) that surrounded highly proliferative tubular complexes (32). Other ECM modifying proteins also secreted by fibroblasts at sites of acute inflammation or injury, including matrix metalloproteinase 2, retinol binding protein 1, and serum amyloid A3, were also up-regulated by quantitative real-time-PCR in this screen. Interestingly, PSCs were first identified as pancreatic resident stromal cells that undergo “activation” after pancreatic injury (39, 40). These activated PSCs expressed the mesenchymal markers vimentin and nestin (41) and demonstrated increased proliferation, migration, and secretion of ECM proteins, including periostin (33). Closely associated with periostin-expressing PSCs were proliferating epithelial cadherin (E-cad^+) ductal tubular complexes (d7) containing single insulin^+ cells or small islet-like clusters (5–10 insulin^+ cells) that emerged by 21 days after pancreatectomy. Therefore, the close proximity of periostin-expressing activated PSCs to proliferating ductal complexes, and their proposed role of periostin in matrix modification and integrin engagement after injury suggested the PSC-periostin axis as a probable intermediate preparing a niche for islet regeneration.

Periostin: A Matrix Bound Factor That Promotes an Islet Regenerative Cascade

The authors used a series of elegant in vivo experiments using periostin null mice (42) to firmly establish the fundamental role of periostin in pancreas regeneration. At 3–5 days after partial pancreatectomy, periostin-deficient mice showed reduced accumulation of stromal cells or PSCs expressing vimentin, collagen 5a and Snail 1, and new ductal tubular complex formation was also decreased as shown by decreased cytokeratin 7 expression. Importantly, after 3 weeks of pancreas regeneration, the emergence of small insulin-expressing β-cell clusters in the regenerating tip was significantly reduced in the periostin-deficient pancreas compared with wild-type mice. Although uninjured periostin-deficient mice demonstrated normal pancreas morphology, blood glucose, and β-cell mass, 1 week after challenge with a single bolus of 100-mg/kg STZ, periostin-deficient mice showed significantly elevated blood glucose, reduced β-cell mass, and reduced serum insulin compared with wild-type controls, which tolerated this modest dose of STZ without reduced endocrine function. Although periostin did not seem to play an essential role in pancreas development, and a potential protective role of periostin on β-cell survival after STZ treatment was not experimentally addressed, periostin was required for efficient recovery of endocrine function after both partial pancreatectomy or STZ administration.

Next, the authors injected 500 ng of recombinant periostin protein directly into the pancreas of wild-type mice and carefully observed a temporal cascade of proliferative events in the pancreas (Figure 1). Using Ki-67 costaining, vimentin-expressing PSC showed a burst of proliferation at 12–24 hours after injection. In contrast, periostin injection did not initially induce proliferation within epithelial cells, islets, or exocrine cells. By day 3, proliferation no longer predominated in the PSC fraction, but proliferation was now found primarily within cytokeratin 7-expressing focal tubular complexes. This second wave of proliferation continued in E-cad^+ cells until day 7, at which time, insulin^+ cells were observed in cytokeratin 7-expressing ductal complex. This pattern of proliferative and differentiative events was similar to that observed in wild-type mice after partial pancreatectomy or after STZ administration. To further support this regen-
erative scheme, at 3 days after periostin injection, the focal tubular complexes associated with PSC accumulation contained numerous E-cad^+/Ki-67^+ that expressed neurogenin 3, a marker of endocrine cell specification (15). Thus, periostin delivered directly into the pancreas was sufficient to initiate a proliferative cascade, characterized by the initial activation and expansion of PSC, followed by enhanced ductal tubule formation and subsequent endocrine cell specification, presumably from Ngn3-expressing facultative endocrine precursors (Figure 1). Implicated in this cascade proposed by the authors is a central role for stromal modification by PSC in the initiation of small islet formation associated with ductal structures, a regenerative mechanism previously reported after the injection of human bone marrow-derived MSC into immunodeficient recipients with STZ-induced β-cell ablation (25–27). Although periostin seems to initiate communication between PSC and neighboring ductal epithelial cells, the specific identity of facultative endocrine precursors or epithelial cells that undergo endocrine specification remains unknown.

Clinical Application: Can Periostin Administration Expand β-Cell Mass?

In long-term studies, wild-type mice that received weekly ip injection of periostin for up to 6 months at increasing concentrations (0–10 μg) showed improved glucose tolerance in response to a glucose bolus, increased pancreas size, and a significant increase in β-cell mass due to an increase in the number of insulin^+ islet clusters. Long-term treatment was well tolerated, and histopathological examination showed that periostin administration had no adverse effects on other organs, suggesting that periostin may have therapeutic potential in the treatment of diabetes. However, the observed regenerative effects after ip injection were modest due to non-specific delivery and potential wash-out. Subsequently the authors developed a novel technique to deliver periostin directly into the ductal tree via injection into the common bile duct in mice previously treated with 125-mg/kg STZ bolus. Astoundingly, mice injected with a single bolus of periostin showed significantly reduced nonfasting blood glucose levels for more than 8 weeks compared with controls similarly injected with mouse serum albumin. Improved glycemia corresponded with enhanced glucose tolerance and increased serum insulin concentrations (Figure 1). Furthermore, detailed histological evaluation at 9 weeks after injection showed a marked increase in the number of insulin^+ islet clusters and total insulin area per section. Remarkably, the vast majority of regenerating islets were small in size (<250 μm^2). Collectively, this definitive experiment clearly established that direct delivery of periostin via the common bile duct-stimulated islet regeneration after STZ-induced β-cell ablation.

Conclusion and Future Directions

The work by Smid et al (32) offers seminal advances to the field of islet regeneration. This manuscript establishes that secretion of periostin by PSC after pancreas damage can initiate a regenerative cascade characterized by the expansion of ductal tubule complexes intimately associated with the emergence of insulin expressing islet-like clusters. Rather than directly inducing the proliferation and expansion of surviving β-cells (11, 30), periostin stimulates endocrine regeneration via an alternate islet neogenic mechanism. Although periostin was not essential for morphologically normal endocrine pancreas development, periostin knockout impaired regeneration specifically within the β-cell compartment in the adult murine pancreas. Thus, developmental mechanisms leading to embryonic or perinatal islet formation may differ significantly from regenerative mechanisms activated after damage in the adult pancreas. Finally, the authors generate “proof of concept” that periostin delivery via the common bile duct was not only well tolerated by murine recipients without adverse events but the periostin protein...
could be delivered directly to the site of islet regeneration, remained bioactive even in the harsh environment of the bile duct, and significantly enhanced small islet formation within 1 week resulting in normalized glycemia that persisted for 8 weeks after injection. Thus, pancreas subcompartment-specific peptide delivery establishes the ductal epithelium as a bonifide niche for islet formation. However, it remains unclear whether common bile duct administration may translate to human application for the specific delivery of peptides or drugs to promote islet expansion during diabetes.

Not unlike other potentially transformative contributions, this work also raises several questions regarding the molecular mechanisms governing new islet formation. First, the molecular events that initially activate PSC after pancreas damage remain unknown. The identification of upstream factors that induce periostin expression would be useful in recapitulating a full pancreatic regenerative response. Next, direct effects of matrix-bound periostin on the formation of proliferating ductal epithelial complexes were not observed by this study. Presumably, additional secreted stimuli from PSC or other associated cell types may add to the complexity of signals required to mediate ductal epithelial cell expansion. Similarly, the identity of facultative β-cell precursors associated with the regenerating ductal epithelium remains controversial (14–16). Future studies are warranted to determine the cell phenotype and associated stimuli that contribute to endocrine specification by ductal-derived precursors. Finally, full functional islet development from the ductal epithelial tree seems truncated in vivo regenerative experimental systems. Single insulin+ cells or small islet-like clusters can emerge, but full large islet formation was not achieved. Likely, additional signals and ECM modifications by PSC, ductal epithelial cells, and other cell types may be absent for the emergence of large functional islets after pancreas damage.

Although inducing islet regeneration using a cocktail of signaling mediators or stem cell administration can now be achieved in experiments systems, it remains unclear whether these regenerative mechanisms are conserved in the adult human diabetic pancreas. Furthermore, the types of regenerative processes elicited seem to be highly dependent on the damage model used. Therefore, the extent of regeneration achieved in the face of ongoing autoimmunity (T1D) or during metabolic toxicity and insulin resistance (type 2 diabetes) will require further preclinical testing. Nonetheless, the potential translation of “endogenous islet regeneration” in situ to the adult human pancreas during diabetes remains an attractive alternative to β-cell replacement therapies.

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