ISLET Neogenesis in Man Without the Need for Transplants

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Abstract

This review demonstrates the role and the data supporting Reg gene peptides in forming new islet containing new populations of beta cells from one's own progenitor cells in the pancreas. Data in man demonstrates that even those patients with 20 years of type 1 diabetes, can generate new islets with Reg gene peptides and during pregnancy when there is upregulation of REG genes. The distinctions between islets in mice and men are also described to provide an understanding of why, in man, there is a unique architecture and vascular of human islets requiring islets to function optimally within the pancreas.

Keywords: REG Gene; Reg (Protein); Reg Peptides; ISLET Neogenesis; Transplants

The Human Genome Project has enabled researchers to discover that the same genes initiating the formation of new islets in fetal development also emerge when the pancreas is injured as a means of protection. More than 70 publications have now demonstrated the role of the regenerating (REG gene) and Reg (protein) family and the efficacy of shorter bioactive Reg peptides to transform progenitor cells within the pancreas into new islets. Human Phase 2B trials have successfully been conducted in both type 1 and type 2 diabetes patients resulting in significant lowering of hemoglobin A1C among type 2 patients and significant rises in stimulated C-peptide, a marker of endogenous insulin production, even among type 1 patients with type 1 for 20 years. Reg peptides provide a completely unique and innovative approach, not requiring transplantation, and having the potential for insulin independence among type 1 and 2 patients, as well as first line theraphyfor diabetes.

In 1920, Moses Barron made the paradoxical observation that pancreatic stones cause islet neogenesis [1]. Barron's observation led Frederick Banting to design his initial studies of ligating the pancreatic ducts in dogs and collecting the remaining pancreatic secretions, which resulted in the discovery of insulin [2,3]. Prior to the widespread availability of insulin, surgeons performed ligations of the tail of the pancreas on diabetic children in the hopes of regenerating islets with demonstration of transient symptomatic improvement [4,5]. For almost a century, the regenerative capacity of the Islets of Langerhans had been well described, but not until the advent of the Human Genome Project is there now supporting data that islet neogenesis can be augmented within patients with diabetes without the use of transplantation and can potentially change the course of the disease known as diabetes.

Whether type 1 or type 2 diabetes or any clinical variant, it is only when the beta cell fails that one develops diabetes. Although there is much in the literature on the role of insulin resistance in type 2 diabetes, insulin resistance does not cause diabetes. Only when the beta cell fails, beyond a tipping point, does diabetes occur. Many people have obesity and insulin resistance, but not until the beta cell can no longer keep to produce enough insulin to keep glucose levels in a normal range, does diabetes occur. Even though we have more than 40 new therapies available for use among type 2 patients and more than 20 insulin preparations available for the treatment of both type 1 and 2 diabetes, none address the underlying cause of diabetes: too few beta cells, which has significant repercussions for the entire islet.

It is critical to understand that there is significantly more complexity of the islets in man, than in mice, which helps explain why so many therapies have been able to reverse diabetes, particularly in type 1 mouse models, and yet these successes cannot be translated into man. When there is beta cell loss, there is initial alpha cell expansion and many other physiological changes that follow, ultimately leading to loss of the complete islet [6-9].

Autopsy studies conducted among both type 1 and 2 diabetes patients demonstrate that not only are there reductions in beta cell numbers, but there are also significant reductions in both the islet numbers and islet mass [6,7]. Thus, not only is there loss of the secretion of insulin and amylin from the beta cell, but also loss of entire islets including alpha cells secreting glucagon, delta cells secreting somatostatin, gamma cells secreting pancreatic polypeptide and epsilon cells secreting ghrelin [6-9]. Each of these hormones play an important and intricate role in glucose homeostasis and gives greater insight into why with new insulin, pump and sensor technology and new therapies, do not provide for glucose cannot be restored to normal levels [10-16]. Sensor data from non-diabetic humans demonstrate that 80% of all measured glucose levels lie within 60 - 100 mg/dL, with mean peak glucose levels after meals of $\leq$ 120 mg/dL [17].

Linear regression curves from the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) show that A1C levels above 5.5% are associated with more complications [18,19]. This data is supported by A1C levels from the EPIC-Norfolk trial among non-diabetic individuals, which found that A1C levels above 5.5% are associated with significantly increased risks for vascular-related morbidity and mortality [20]. The data in healthy subjects underscores why it is so critical to have healthy functional islets for glucose and A1C levels to remain within the normal range.

The "Bionic Pancreas," which delivers both insulin and glucagon to patients with a computer algorithm that does not require manual adjustments by the patient, yet does not restore glucose levels into the normal range [21]. Too often, in the field of diabetes, we label patients as being, "noncompliant," it is clearly the pancreas that is "noncompliant". By the time diabetes is diagnosed, the beta cell tipping point has been exhausted. Diabetes is an escalating and devastating global epidemic because none of the current therapies have addressed the underlying etiology of diabetes: too few functioning beta cells.

Differences between human and rodent islets

In recent years, human islet structure and cellular organization have been carefully scrutinized, leading to new questions and controversies regarding the differences between islets in man and mouse. While there are some similarities, the composition, architecture, innervation, and function of human islets are quite different than those seen in mice [22-26], human beta cells often are not the only cell clustered in the center of an islet as they are in rodents. In contrast to mice, some studies have demonstrated that more than 70% of human beta cells have direct physical associations with other endocrine cells (e.g. alpha, delta, and gamma/pancreatic polypeptide cells), suggesting that unique paracrine interactions may occur between beta cells and their immediate neighbors [22].

There is clearly a more complex arrangement with many different islet profiles in humans and other primates [22-29].

Although there appears to be heterogeneity among human islets, studies have shown that compared to rodent islets, those in human and nonhuman primates have increased proportions of alpha and delta cells and are more dispersed throughout the islet, with lower proportion of beta cells [22-27]. Furthermore, rodent islets have fewer alpha and delta cells relative to beta cells, and these are primarily found in the islet periphery [22-27]. Human and nonhuman primate islets have better developed and more prominent internal vasculature than rodents [22-27].
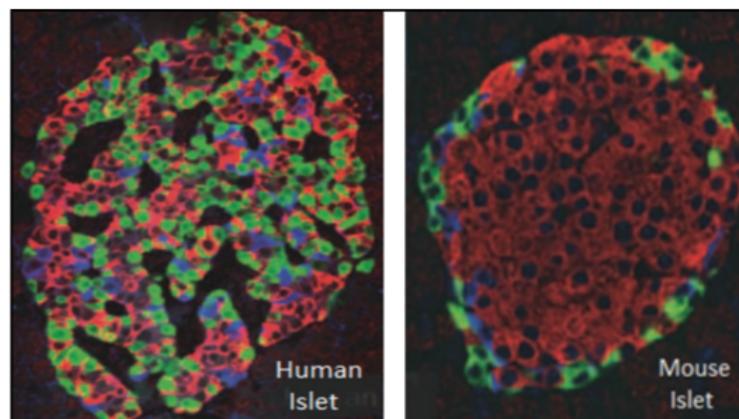
Figure 1: *Architecture of mouse and human islets. The above images demonstrate differences between islets in men and mice. Insulin staining is in red, glucagon in green, and somatostatin in blue. The black areas distinctly seen within the human islet are occupied by blood vessels that are not seen in mice. The blood vessels within the islets are lined by smooth muscles innervates by the autonomic nervous system, which allows for regulation of blood glucose levels in the human islet not seen in the mouse islet. Reprinted with permission, Copyright 2006, Proceedings of the National Academy of Sciences of the United States of America. 2006; 103(7): 2334-2339.*

The blood vessels within the human islet contain a larger proportion of smooth muscle cells, which has implications for sympathetic nervous system innervation. Conversely, rodent islet vasculature consists mainly of endothelial tubes devoid of smooth muscle cells that occupy a smaller physical space within the islets [22-26].

These distinctions help explain why human islet transplants from cadaveric pancreata that are injected via the portal vein and rests in the liver and studies have transplants islets into the abdominal cavity have not been successful long term, due to the lack of sustainability of islets outside the pancreas.

These findings are underscored by the juxtaposition of functioning beta cells is critical for glucagon regulation from alpha cells [30]. Without appropriate intra-islet insulin and amylin secretion from beta cells, there is aberrant feedback to alpha cells, resulting in unregulated glucagon hypersecretion, which may directly lead to diabetic symptoms [31].

Islet neogenesis without islet transplantation in man

Teams from around the world have more recently shown, just as physicians recognized nearly a century ago, that acute pancreatic injury results in the formation of new islets from progenitor cells found in the pancreatic ductal population [32-35]. With the advent of the Human Genome Project, the regenerating gene (REG) and regenerating gene protein (Reg) family has emerged among more than a dozen mammalian species, including man, as a key initiating factor in the process of islet neogenesis [36-57].

Despite islets only comprising 2% of the islet mass, islets receive 20% of the blood flow to the pancreas. Islet neogenesis involves generation of whole new islets containing all five cell types, not just the beta cell secreting insulin and amylin, but also the other four cell types each secreting hormones which are intricately involved in glucose homeostasis.

In humans, the Reg genes are typically expressed only during fetal development when islets are formed for the first time, but are up-regulated as a protective mechanism, when there is acute pancreatic injury to initiate the formation of new islets.

Additionally, the Reg gene has also been shown to be upregulated during pregnancy and pancreatitis [58-63].

The Reg gene proteins are a family of C-type lectin proteins that are expressed by the pancreas. A Reg knockout mouse model has also demonstrated the important role of Reg genes in glucose homeostasis with diminished ((3)H)thymidine incorporation in isolated islets from Reg knockout mice, and hyperplastic islets were induced by the injection of gold thioglucose with the average islet size in Reg knock-out mice being significantly smaller than that of control Reg(+/+) mice [64]. The ability to translate this genomic science into therapeutics has been shown by the discovery and efficacy of the shorter bioactive peptide regions of the Reg gene proteins that transform pancreatic cells into new islets in mammals including humans [65-75].

Islet neogenesis during pregnancy

The potential for patients with longstanding type 1 diabetes to develop new endogenous insulin production is consistent with the work of Jovanovic and colleagues who have demonstrated that within 10 weeks of pregnancy, among consecutive patients with type 1 diabetes for an average of 20 years, that there is a rise of C-peptide into the normal range [76]. This can be hypothesized due both the expression of Reg during pregnancy [64] and other growth factors contributing to islet neogenesis as well as the suppression of the mother's immune system to protect the fetus from autoimmune attack since the fetus has 50% differing DNA from the mother, which diminishes attack on beta cells.

(with permission of Dr. Lois Jovanovic   Diabetologia 2000: 43;1329-1336)
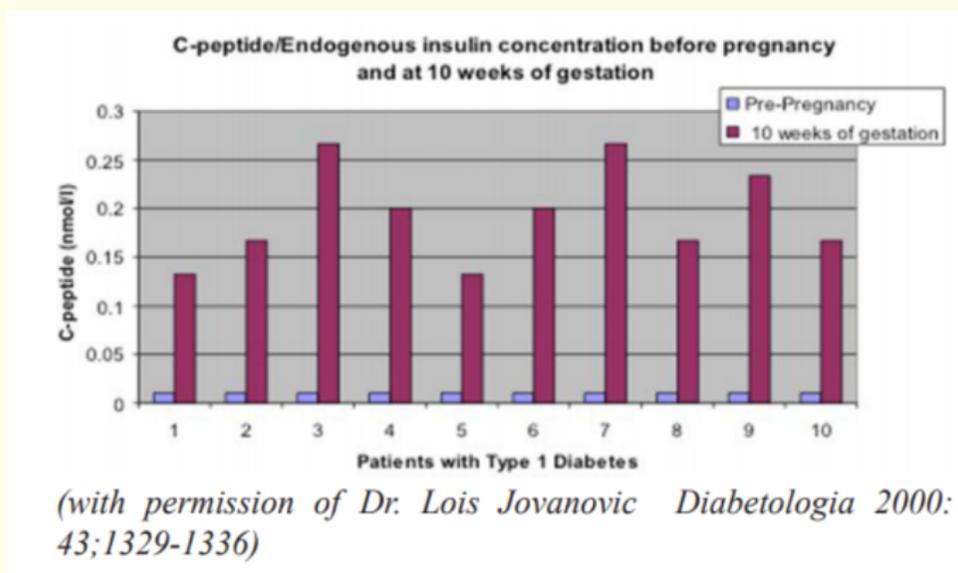
Figure 1: C-peptide rise among ten consecutive pregnant type 1 patients with a mean duration of diabetes for 21.2 years. C-peptide levels were measured before pregnancy in the fasting state and at 10 weeks of pregnancy. There was a rise in C-peptide concentration from a non-detectable concentration pre-pregnancy to a mean concentration of 0.58 ng/ml (0.2 nmol/l) at 10 weeks of gestation; REG genes having been shown to be upregulated in pregnancy [76].

Efficacy of short Reg peptides in islet neogenesis

These shorter Reg gene peptides (Islet Neogenesis Associated Protein, a 15 amino-acid sequence within the hamster Reg 3 gamma gene protein, referred to as INGAP and Human proIslet Peptide/HIP, a 14 amino-acid sequence within the human Reg3a gene protein have been shown as potential therapeutic agents in type 1 and 2 diabetes. Shorter Reg peptides have been shown in both type 1 and 2 diabetes mouse models to reverse diabetes and *in vitro* studies to transform human ductal tissue into islets.

Most importantly, randomized double-blind controlled trials studies have been conducted in man through human phase 2B trials with the demonstration among type 1 diabetes patients with a mean of 21 years of the disease having a 27% rise in C-peptide area under the curve by day 54 of treatment (p = 0.0057) [77]. Among type 2 patients, Reg peptides have been used in human clinical Trials, INGAP and HIP demonstrated a potential to improve diabetes, including a significant reduction in A1C [77-80].

Reg gene peptides have been shown to upregulate transcription factors including PDX-1, NGN3 a marker of islet progenitors, Neu-rodD1, Pax4, MafA, Nkx2.2, Nkx6.1, B4n4, MafB, Pax6,Nkx6.1 and Sox9, which are also stimulated by the shorter Reg peptides found within the binding region above and acting through the Reg receptor [65,67]. In a study evaluating the presence of Reg peptide in newly forming islets directly budding from pancreatic exocrine ducts, staining for Reg peptide was found to be highly expressed in newest islet clusters just budding from exocrine ducts, again supporting the important role of Reg in transforming ductal progenitors to islets [81].

Distinctions between islet neogenesis and beta cell regeneration

Whenever discussing Reg and its role in diabetes treatment, it is important to distinguish between beta cell regeneration from existing beta cells and the genomic processes of islet neogenesis from ductal progenitors. The terms "beta cells" and "islets" are often, in error, used synonymously, even in the basic science literature. In man, beta cells must live within the islet, where their borders can be contiguous with the alpha, delta, epsilon and gamma cells, and islets most optimally function within the pancreas, where the islet mass gets a disproportionate amount of the blood supply to the pancreas. The genomic differences between islet neogenesis and beta cell replication have been demonstrated in a review by Levetan [82].

Studies have shown that human pancreatic ductal progenitor cell can be transformed into new islets in the presence of the shorter Reg peptides. Reg peptides represent a new therapeutic class of in the diabetes armamentarium, known as islet neogenesis agents. Reg peptide therapy holds true promise and the key to a future without diabetes as we know it today. The key to success in using these new Reg therapies is to generate new islets at a greater rate than destruction of beta cells within islets. The challenges among both type 1 and 2 diabetes are to maintain new islets containing new pools of beta cells and protect them from destruction; whether that be autoimmune attack in the case of type 1 diabetes or the multiple factors in type 2 leading to a tipping point in which the beta cells cannot generate enough insulin to maintain glucose levels in a normal range.

There are studies in both type 1 and type 2 diabetes demonstrating how to protect new insulin producing cells from destruction. Among type 1 diabetes, which is an autoimmune disease, thirty years of trials utilizing various immune suppressing agents and therapies that act as biologic response modifiers have all shown great promise of generating immune tolerance to the autoimmune attack on beta cells, in mice but not in man. In contrast to type 1 mouse models, in man, even in an immune muted milieu, new beta cells are not generated at a rate to result in sustained insulin independence, thus, like in patients who receive islet transplants, immune therapy will also be needed among type 1 patients with Reg peptide therapy.

Regeneration of the pancreatic islets is not only a dream, but also has demonstrated efficacy in type 1 diabetes patients. Generating new islets within the pancreas and beta cells within the islets may be the future of reversing diabetes. The following was presented at a recent International Summit on Insulin Independence [83].
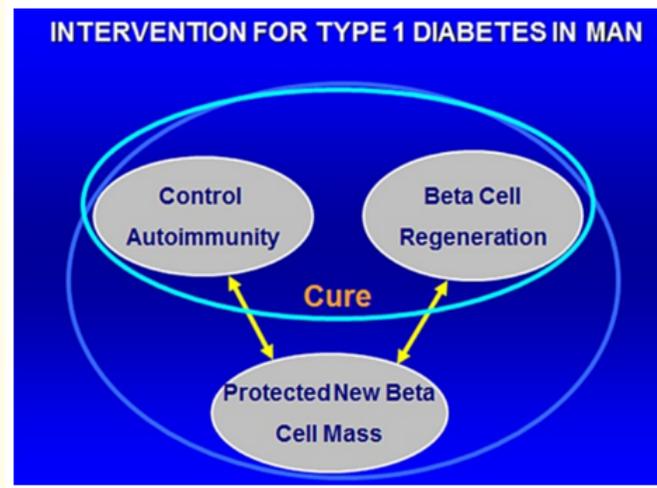
Figure

Summary

More than 700 studies have been published on the role of the Reg gene proteins and shorter Reg peptides. Human trials through phase 2B have demonstrated beneficial results among both type 1 and 2 patients with diabetes with the ability of Reg peptides to significantly increase C-peptide among patients with type 1 diabetes for more than 20 years.

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