GAVIN PUBLISHERS

Journal of Diabetes and Treatment

Levetan C. J Diabetes Treat 2017: J116.

Review Article

Islet Neogenesis Therapy from Endogenous Pancreatic Stem Cells without Transplantation: Will Type 1 and Type 2 Diabetes Soon be Diseases of the Past?

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Citation: Levetan C (2017) Islet Neogenesis Therapy from Endogenous Pancreatic Stem Cells without Transplantation: Will Type 1 and Type 2 Diabetes Soon be Diseases of the Past?. J Diabetes Treat 2017: J116.

Received Date: 28 March, 2017; **Accepted Date:** 20 April, 2017; **Published Date:** 27April, 2017

Abstract

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 in 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.

Diabetes Past

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 insulin2,3 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]

Thus, 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 noegenesis can be augmented within patients with diabetes without the use of transplantation and can potentially change the course of the disease known as diabetes.

Diabetes Present

Even though we have more than 30 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 manyother 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 us greater insight into why even

with all of the current therapies available today, including technologies like glucose sensors and insulin pumps, 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 <120 mg/dL. [17]

A presentation at the 2016 American Diabetes Association Scientific sessions pointed out that despite all of the new diabetes therapeutics, among 70,657 patients with diabetes, the proportion of patients able to maintain their A1C below 7% has not increased to beyond 40% since 2008. [18] 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.[19-20] 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. [21]

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. Even the "Bionic Pancreas," which delivers both insulin and glucagon to patients with a computer algorithm that does not require manual adjustments by the patient, does not restore glucose levels into the normal range. [22]

Too often, in the field of diabetes, we label patients as being, "noncompliant," when in the field of diabetes; it is clearly the pancreas that is "noncompliant." By the time diabetes is diagnosed, the beta cell tipping point has been exhausted. We can better understand why diabetes is an escalating and devastating global epidemic because none of the current therapies until now, have addressed the underlying etiology of diabetes.

The International Diabetes Federation estimates that there are 415 million adults living with diabetes worldwide and this number could rise to 642 million by 2040. Although most patients with the disease have type 2 diabetes, type 1 comprises 7-12% of all diabetes cases worldwide (29-50 million people) and the incidence of type 1 diabetes is increasing at a rate of 3% per year.We can better understand why diabetes is an escalating and devastating global epidemic because none of the current therapies until now, have addressed the underlying etiology of diabetes.

The Diabetes Future

Scientific 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.[23-39] 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. [40-66] In humans, the Reg genes are typically expressed only during fetal development when islets are formed for the first time, but are upregulated 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. [67-74]

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 goldthioglucose with the average islet size in Reg knockout mice being significantly smaller than that of control Reg(+/+) mice. [75]

The ability to translate this exciting genomic science into therapeutics has been shown by the discovery and efficacy of the shorter bioactive peptide regions of the Reg gene proteins. [76-120] These shorter Reg gene peptides (**I**slet **N**eogenesis **A**ssociated **P**rotein/**INGAP**, **H**uman pro**I**slet **P**eptide/**HIP**, **P**eptides **H**ealing **I**slets of **L**angerhans/**PHIL**) have been shown as potential therapeutic agents in type 1 and 2 diabetes.

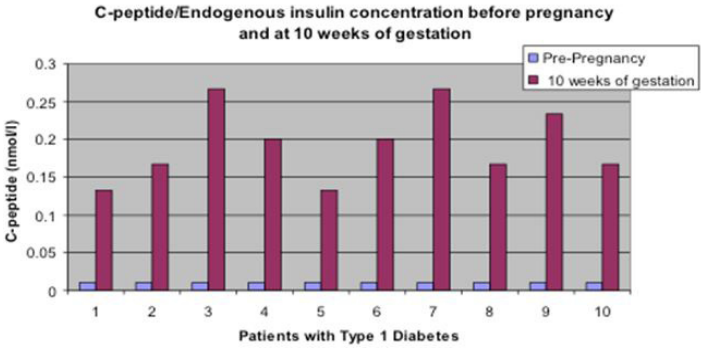
Shorter Reg peptides have been shown in both type 1 and 2 diabetes mouse models to reverse diabetes and *in vitro* studies to transform human ductal tissue into islets, and most importantly, studies have been conducted in man through human phase 2B trials. [76-77] , [118-120] Among type 2 patients, Reg two peptides have been used in human clinical Trials, INGAP and HIP demonstrated a potential to improve diabetes.[118-120] Among type 1 diabetes patients who have had the disease for 20 years, there was a significant rise in stimulated C-peptide (27%; p=0.0057), when INGAP was used subcutanesouly at a dosage 600 mg, within 8 weeks of treatment. [118-120]

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. [123] For example, 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.

Studies have shown that human pancreatic ductal progenitor cell can be transformed into new islets in the presence of the shorter Reg peptides. [76-77] Reg gene proteins have been shown to up-regulate transcription factors including PDX-1, NGN3 a marker of islet progenitors, NeurodD1, 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. [68,76,78] 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. [121]

This data highlights the potential for patients with longstanding type 1 diabetes to develop new endogenous insulin production, and 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. [122] This can be hypothesized in part, due to the expression of Reg 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.



(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 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. To date, the bioactive region of the Reg gene proteins have been identified in man and 17 other mammals. When a bioactive Reg peptide was labeled and injected into a mammal, it was exclusively found to bind in pancreatic ductal tissue, where progenitor cells reside and not found in any other organ. [89]

The figure below demonstrates the region on the Reg gene proteins, which have been identified as the binding region to the Reg gene receptor that have been successfully used as novel therapies in both type 1 and 2 diabetes.

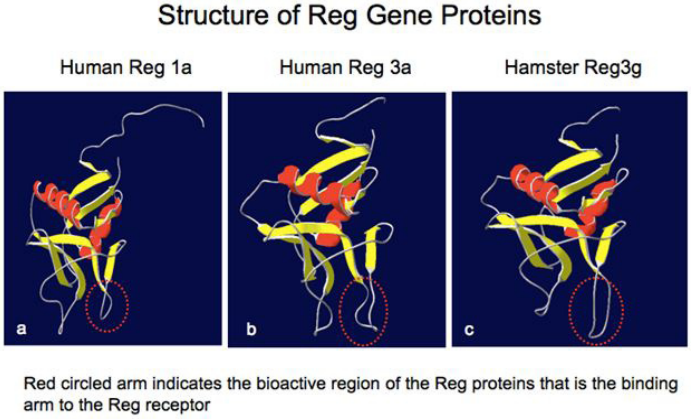


Figure 2: Three-dimensional modeling of the mammalian Regprotein sequences by SwissProt folding algorithms reveal that the binding region sequence is presented and exposed on the external surface of the protein and not folded within the confines of the protein; thus, it is available for protein-binding interactions with the Reg receptor.

With Reg peptide therapies, the potential now exists to address the underlying cause of diabetes; too few beta cells and subsequent loss of islets, which neither insulin nor any current diabetes therapies on the market addresses. 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 challenge among both type 1 and 2 diabetes is 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, there is data to support biologic response modifiers that have a high safety and efficacy profile for protection of new endogenous insulin production . Early intervention with Reg therapy in those at risk for diabetes, may potentially prevent diabetes. In those already diagnosed with type 2 diabetes, there are many therapeutic options and lifestyle modifications to prevent beta cell apoptosis once new beta cell populations are restored with Reg therapies.

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.

This calls for the potential usage a biologic response modifier in combinationwith Reg peptide therapy among type 1 patients. One unique consideration is a biologic response modifier such as oral interferon alpha which has been dosed at 1/1000 of the dosage used to treat hepatitis C, hairy cell leukemia and was shown to be effective in preventing diabetes in NOD mice and safely given to new onset type 1 diabetes in 3 human trials among new onset type 1 patients treated for 12 months with the ability for the treatment arm to maintain more beta cell mass than placebo. [124 -128] The future for using this, or another biologic response modifier, will be a new combination therapy approach, one which combines an agent to change the autoimmune response with a Reg agent to generate new islets.

There is now tremendous excitement in the field of type 1 diabetes to both generate new islets and protect new insulin-producing cells from autoimmune destruction. The use of Reg peptide therapy among type 2 diabetes is an entirely new approach and may also be used to prevent type 2 diabetes among those who have pre-diabetes. As shown in the TODAY Study among children and adolescence with type 2 diabetes, the majority of those diagnosed with diabetes, despite treatment with oral therapy and lifestyle modification go on to require insulin. [129]

Due to lack of beta cells within a functional islet, even with all of the new therapies and insulins available, diabetes not only remains the leading cause of blindness, amputations and kidney failure requiring dialysis or transplants, but also the rates of life-threatening hypoglycemia requiring hospital admission now exceed those for hyperglycemia among older adults in the United States. [130]

Summary

There is a desperate need for new therapies that address the underlying cause of diabetes. The advent of the Human Genome Project has provided a unique window for researchers in the field of regenerative medicine to develop therapies that can utilize progenitor cells in each organ that are present in times of acute injury to regenerate new healthy cells. Reg peptide therapy may not only be another treatment for diabetes, but also provide a completely unique and innovative approach, which addresses the underlying cause of the disease. Reg peptide therapy has the potential to be another break through therapy as insulin was a century ago. With research expanding beyond immunotherapy to include islet regeneration therapy, the dismantling of both type 1 and 2 diabetes is upon us.

ACKNOWLEDGMENTS

I gratefully acknowledge Patrice Cocco and Susan Pierce for their courage and passion to change this disease and all of my patients with diabetes who are truly an inspirational force.

Disclosures: Dr. Levetan is a shareholder in Perle Bioscience and CureDM Holdings.

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