

# Proposal for generating new beta cells in a muted immune environment for type 1 diabetes

Claresa Levetan\*

Paolo Pozzilli

Lois Jovanovic

Desmond Schatz

*Department of Endocrinology,  
Chestnut Hill Hospital, Philadelphia,  
PA, USA*

\*Correspondence to: Claresa Levetan,  
Department of Endocrinology,  
Chestnut Hill Hospital, 8815  
Germantown Avenue, Suite 35,  
Philadelphia, PA 19118, USA.  
E-mail: ResaLevetanMD@gmail.com

## Abstract

**Background** Over the past decade, many immune tolerance agents have shown promise in the non-obese diabetic mouse model for prevention and reversal of type 1 diabetes but have not been successful in clinical trials among recently diagnosed type 1 patients. The trials from decades ago using Cyclosporine A in significantly lower dosages than used for organ transplantation and in similar dosages that have increased T regulatory cell populations in conditions such as atopic dermatitis, demonstrated very high initial insulin-free remission rates when administered immediately after diagnosis. Over time, all newly diagnosed type 1 patients given Cyclosporine A required insulin. Human trials with immune tolerance agents suggest that in addition to an immune tolerance agent, a beta cell regeneration agent may also be necessary to induce long-lasting remission among patients with recent onset type 1 diabetes.

**Methods** A randomized, double-blind prospective trial among recent onset type 1 diabetes patients has been designed using Cyclosporine A and a proton-pump inhibitor, which increases gastrin levels and has been shown to work through the Reg receptor to transform pancreatic duct cells into islets. © 2013 The Authors. *Diabetes/Metabolism Research and Reviews* published by John Wiley & Sons, Ltd.

**Keywords** type 1 diabetes; immune protection; beta cell regeneration

## Making peace with the immune system is not enough for type 1 diabetes in humans

Over the past three decades, there have been several trials using immune tolerance agents demonstrating both prevention and reversal of type 1 diabetes in non-obese diabetic mice, yet these results have not been translated into man. As we look at recent clinical trials among patients using immunotolerance agents, including anti-CD3 antibodies, anti-CD20 monoclonal antibody, interleukin 1-receptor antagonists, anti-thymoglobulin and others that have reversed diabetes in type 1 mouse models, the insulin-free remission rates in humans have not been seen. It is now clear that unlike the diabetes remissions seen in rodents, making peace with the immune system is not enough to generate new beta cells in humans.

As we begin to understand that autoimmunity is only one aspect of type 1 diabetes in humans and the other being beta cell deficiency, we can now look at the efficacy of the immune tolerance agents in a new light. Lasting insulin-free

remissions in humans will only occur when beta-cell mass is sustained and regenerated over time in the milieu of immune protection.

If we now examine the efficacy of immune tolerance agents based on insulin-free remissions at 1 year following initiation, some of the most promising data actually dates back 25 years. Cyclosporine A had a 67.5% insulin-free remission among recent onset type 1 patients, with 50% of patients sustaining the insulin-free state after 12 months [1]. However, by four years, all patients required insulin [2]. Renal side effects were not seen in the many trials, including a published cohort of 285 patients with recent type 1 diabetes followed for up to 13 years after 20 months of therapy on cyclosporine [3]. The usage of Cyclosporine A among patients with dermatological conditions has been associated with significantly increased T regulatory cells, both in absolute numbers and frequencies and in the reduced ability of T cells to be activated [4].

Despite immune intervention, the inability to generate new beta cells has contributed to the necessity for exogenous insulin. Distinct complexities of human islets, including the slower beta-cell turnover rates in humans compared with mice, may account for the need for the use of a beta cell regeneration agent in addition to an immunosuppressive or immunoregulatory agent when intervening in the disease [5].

### Beta regeneration therapies utilized in humans

Novel therapies have emerged with a mechanism of transforming ducts into islets. For example, the Reg3 gene proteins, which have been found to be upregulated in acute pancreatic ligation and in new onset human type 1 diabetes, and have been shown to generate new islets from progenitor cells found within the pancreatic ductal population. *In vitro* studies have shown Reg peptides transform human pancreatic ducts to islets [6,7]. A recent BrdU study found that two Reg peptides resulted in a twofold increase in the volume of small new islets after 5 days of treatment ( $p < 0.05$ ) [8]. Five days of Reg peptide treatment also demonstrated upregulation of pancreatic transcription factors, including Ngn3, Nkx6.1, Sox9 and Ins. The Reg3 gamma peptide, islet neogenesis associated protein, demonstrated a 27% rise in arginine stimulated C-peptide area under the curve among 63 type 1 patients, with an average of 20 years of type 1 diabetes with no prior detectable fasting C-peptide levels ( $p = 0.0057$ ) [9]. Three Reg peptides are currently in development.

By similar mechanisms of action to the Reg peptides, gastrin has been shown to stimulate islet cell hyperplasia [10]. Preliminary studies combining gastrin with epidermal growth factor resulted in up to a 75% reduction in insulin requirements among existing type 1 diabetes patients

[11]. Proton pump inhibitors have been shown to increase gastrin levels ninefold in man. A recent study among type 2 diabetes patients demonstrated that the proton pump inhibitor, pantoprazole, given at dosage of 40 mg twice daily for 12 weeks resulted in a decrease in A<sub>1C</sub> of 7.6–6.8% ( $p < 0.001$ ). The decrease in A<sub>1C</sub> significantly correlated with an increase in gastrin and insulin levels and beta-cell function assessed by Homeostatic model assessment [12].

Thus, in type 1 diabetes, therapeutic agents that may generate new islets from ductal tissue may hold great promise when used in conjunction with an immune tolerance agent. Such an approach may potentially increase beta-cell mass among type 1 patients in a muted immune environment, in order to protect and sustain both remaining beta cells and newly generated beta cells.

### Distinctions between islets in mice and humans

In mice, type 1 diabetes is primarily an autoimmune disease, and rendering mice insulin independent is different than it is for humans. Rodents have a greater capacity to regenerate beta cells faster than in humans. Contributing factors may include their continuous patterns of eating, and the fact that their islets have a higher percentage of beta cells and lower percentage of alpha, delta, pancreatic polypeptide and ghrelin-secreting epsilon cells. Rodents also have less complex vascular, neural and paracrine systems. Perhaps, these differences and complexities help explain why diabetes can be ‘cured’ in a mouse with immunosuppression alone but not in humans, in which the reversal of diabetes requires islet regeneration in addition to immune protection.

These studies posit that, in humans, type 1 diabetes is both an autoimmune disease and a disease of beta-cell deficiency, which cannot be corrected with immune agents alone, as illustrated by the diagram below (Figure 1).

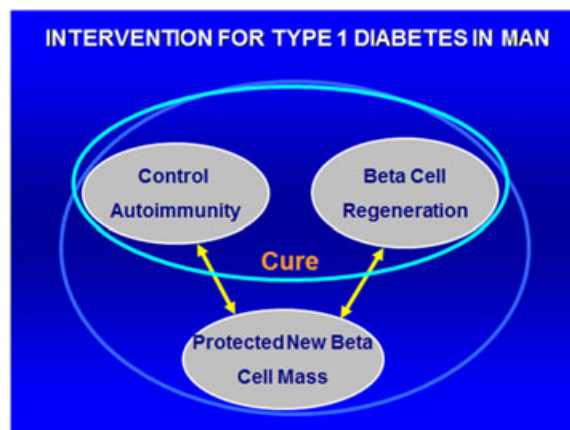


Figure 1. A novel proposal to tackle type 1 diabetes at diagnosis

## Need for combination therapy for type 1 diabetes

Because type 1 diabetes is both an autoimmune disease and a beta-cell deficiency disease, it cannot be corrected with immune dampening agents alone. Our analysis of the shortcomings of the earlier therapeutic approaches to type 1 diabetes have led us to design a new proposal, utilizing the immune muting agent cyclosporine with the proton pump inhibitor, lansoprazole, a drug that markedly

enhances plasma levels of gastrin. Gastrin has been associated with the *in vivo* expansion of islet neogenesis from ductal populations acting through the Reg receptor [10,13,14].

With this in mind we are now starting a novel trial <http://www.clinicaltrials.gov/ct2/show/NCT01762644?term=IIT&rank=1> utilizing the immune agent Cyclosporine A with the proton pump inhibitor, lansoprazole. Each of the agents has a long enough track record so that the anticipated risks of the study are modest.

## References

1. Bougneres PF, Carel JC, Castano L, *et al.* Factors associated with early remission of type I diabetes in children treated with cyclosporine. *N Engl J Med* 1988; **17**;318(11): 663–6670.
2. De Filippo G, Carel JC, Boitard C, *et al.* Long-term results of early cyclosporin therapy in juvenile IDDM. *Diabetes* 1996; **45**(1): 101–104.
3. Assan R, Blanchet F, Feutren G, *et al.* Normal renal function 8 to 13 years after cyclosporin. A therapy in 285 diabetic patients. *Diabetes Metab Res Rev* 2002; **18**(6): 464–472.
4. Brandt C, Pavlovic V, Radbruch A, Worm M, *et al.* Low-dose cyclosporine. A therapy increases the regulatory T cell population in patients with atopic dermatitis. *Allergy* 2009; **64**(11): 1588–1596.
5. Levetan CS, Pierce SM. Distinctions between the islets of mice and men: implications for new therapies for type 1 and 2 diabetes. *Endocr Pract* 2013; **19**(2): 301–312.
6. Li J, Wang Y, Yu X, Chen H. Islet neogenesis-associated protein-related pentadecapeptide enhances the differentiation of islet-like clusters from human pancreatic duct cells. *Peptides* 2009; **30**(12): 2242–2249.
7. Levetan CS, Upham LV, Deng S, *et al.* Discovery of a human peptide sequence signaling islet neogenesis. *Endocr Pract* 2008; **14**(9): 1075–1083.
8. Kapur R, Højfeldt TW, Mogensen JP. Short-term effects of INGAP and Reg family peptides on the appearance of small b-cells clusters in non-diabetic mice. *Islets* 2012; **4**(1): 1–9.
9. Dungan KM, Buse JB, Ratner RE. Effects of therapy in type 1 and type 2 diabetes mellitus with a peptide derived from islet neogenesis associated protein (INGAP). *Diabetes Metab Res Rev* 2009; **25**: 558–565.
10. Wang TC, Bonner-Weir S, Oates PS. Pancreatic gastrin stimulates islet differentiation of transforming growth factor a-induced ductular precursor cells. *J Clin Invest* 1993; **92**: 1349–1356.
11. (Transition Therapeutics, March 5, 2007 <http://www.transitiontherapeutics.com/media/archive.php> Accessed January 1, 2013).
12. Singh PK, Hota D, Dutta P. Pantoprazole improves glycemic control in type 2 diabetes: a randomized, double-blind, placebo-controlled trial. *J Clin Endocrinol Metab* 2012; **97**(11): E2105–E2108.
13. [http://string-db.org/version\\_9\\_05/newstring.cgi/show\\_network\\_section.pl?all\\_channels\\_on=1&interactive=yes&limit=0&network\\_flavor=evidence&targetmode=proteins&identifiers=10116.ENSRNOP00000019863%250D10116.ENSRNOP00000054678%250D10116.ENSRNOP00000018583#.UdHgre7uO9c.gmail](http://string-db.org/version_9_05/newstring.cgi/show_network_section.pl?all_channels_on=1&interactive=yes&limit=0&network_flavor=evidence&targetmode=proteins&identifiers=10116.ENSRNOP00000019863%250D10116.ENSRNOP00000054678%250D10116.ENSRNOP00000018583#.UdHgre7uO9c.gmail)
14. [http://stitch.embl.de/version\\_3\\_1/cgi/show\\_network\\_section.pl?identifiers=10116.ENSRNOP00000054678%250D10116.ENSRNOP00000019863&all\\_channels\\_on=1&interactive=no&network\\_flavor=confidence&targetmode=proteins](http://stitch.embl.de/version_3_1/cgi/show_network_section.pl?identifiers=10116.ENSRNOP00000054678%250D10116.ENSRNOP00000019863&all_channels_on=1&interactive=no&network_flavor=confidence&targetmode=proteins)