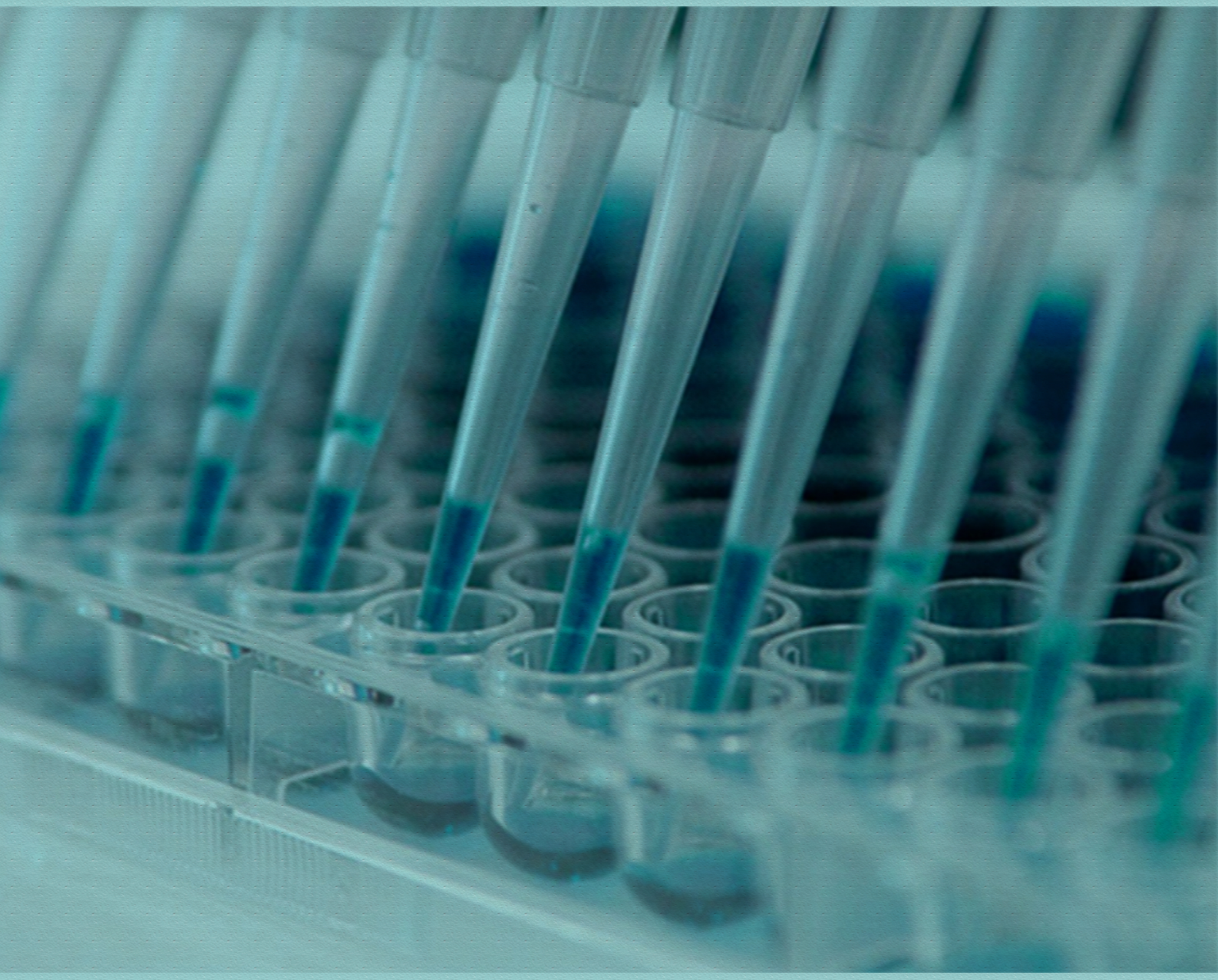
July, 2015 • Volume 1, Issue 3

Openventio PUBLISHERS

ISSN 2379-6391

DIABETES RESEARCH

Open Journal



www.openventio.org

DIABETES RESEARCH

Openventio PUBLISHERS

ISSN 2379-6391

Open Journal

TABLE OF CONTENTS

DIABETES RESEARCH

Openventio PUBLISHERS

ISSN 2379-6391 Open Journal http://dx.doi.org/10.17140/DROJ-1-109

Mini Review

Corresponding author
Naresh Sachdeva, PhD
Assistant Professor
Department of Endocrinology
Post Graduate Institute of Medical Education and Research (PGIMER)
Chandigarh - 160012, India
Tel. +91-172-2755282
Fax: +91-172-2744401
E-mail: naresh_pgi@hotmail.com

Volume 1 : Issue 3
Article Ref. #: 1000DROJ1109

Article History
Received: May 15th, 2015
Accepted: June 8th, 2015
Published: June 10th, 2015

Citation
Paul M, Jacob N, Sachdeva N. Regulatory T cells in treatment of type-1 diabetes: types and approaches. *Diabetes Res Open J*. 2015; 1(3): 54-66. doi: 10.17140/DROJ-1-109

Regulatory T Cells in Treatment of Type-1 Diabetes: Types and Approaches

Mahinder Paul, Neenu Jacob and Naresh Sachdeva*

Department of Endocrinology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh-160012, India

ABSTRACT

Regulatory T cells (Tregs) play important role in regulation of immune responses to self-antigens. Alterations in frequency and function of Tregs have been reported in Type 1 Diabetes (T1D) subjects. Tregs have the potential to prevent destruction of pancreatic beta cells by targeting effector T cells (Teff) and other immune cells causing inflammation. Therefore, strategies to increase Treg cell numbers and function are being explored as potential immunotherapeutic approaches in treating T1D. Today, several groups are involved in exploring different Treg cell types, sources, induction procedures and experimental systems in pursuit of generation of highly efficacious and stable Tregs for their clinical applications. Various protocols have been developed for the induction and expansion of islet antigen specific Tregs and polyclonal Tregs. Studies have shown that antigen specific Tregs are required at less number and are more efficient than polyclonal Tregs in suppressing autoimmune diabetes and they do not cause generalized immune suppression. Alternatively, generation of colonic Tregs (cTregs) has also gathered attention in recent years as an approach to limit pancreatic inflammation *via* gut induced tolerance. With a definitive treatment for T1D still elusive, application of Tregs as a part of combination therapy seems promising in treatment of T1D.

KEYWORDS: Type-1 Diabetes; Regulatory T Cells; Autoimmune diabetes; Immune suppression; Antigen specific.

ABBREVIATIONS: Tregs: regulatory T cells; cTregs: colonic Tregs; Teff: effector T cells; T1D: Type 1 Diabetes; CTL: Cytotoxic T Lymphocyte; T_H: T Helper; GITR: Glucocorticoid-induced TNF- receptor; APC: Antigen Presenting Cell; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; Nrp-1: Neuropilin 1; FoxP3: Forkhead box P3; PLN: Pancreatic Lymph Nodes; DCs: Dendritic Cells; GALT: Gut Associated Lymphoid Tissue; GILZ: Glucocorticoid-induced leucine zipper; TT: Tetanus Toxoid; ATRA: All Trans Retinoic Acid; nTregs: natural Tregs; iTregs: induced Tregs; ASF: Altered Schaedler Flora; TSDR: Treg Specific Demethylated Region; PDL1: Programmed Death Ligand 1; fHASC: human amniotic fluid stem cells; IFA: Incomplete Freund's Adjuvant; LAP: Latency- associated peptide; GARP: Glycoprotein A Repetition Predominant; PSA: Polysaccharide A; MLN: Mesenteric Lymph Nodes; SCFA: Short Chain Fatty Acids; Aldh1a: Aldehyde dehydrogenase; Dapl1: death-associated protein like 1; Igfbp4: insulin-like growth factor binding protein.

INTRODUCTION

Type 1 Diabetes (T1D) is mainly a T cell mediated autoimmune disease characterized by the destruction of pancreatic beta (β) cells leading to insulin deficiency. Regardless of the predisposing factors and environmental triggers, the main pathogenic mechanism leading to T1D is the priming of CD8+ T cells by the autoreactive CD4+ T cells. These CD8+ T cells further recognize and destroy pancreatic β cells by releasing cytotoxic granules mainly containing granzymes and perforin molecule.[1] Such autoreactive CD8+ T cells can be easily detected from the peripheral blood of T1D subjects, as they are more differentiated and express central memory markers.[2-4] In healthy individuals these autoreactive T cells are either eliminated in

thymus or suppressed by regulatory T cells (Tregs) in the peripheral circulation.

Regulatory T cells

These cells also called as suppressor T cells, are a subpopulation of T cells that play an important role in regulation of exaggerated immune response to self/foreign antigens.[5] Tregs are important in induction and maintenance of self-tolerance.[6] They comprise 1-10% of the T Helper (T_H) cell population in healthy adult humans and mice.[5,7,8] These cells express high levels of surface marker CD25, Forkhead box P3 (FoxP3)[9] along with low CD127[10-12] which together have been suggested as reliable markers for Tregs. Tregs have the capacity to actively block immune responses, inflammation and tissue destruction by suppressing the functions of various cell types and processes, including classical T_H cells, B-cell antibody production, affinity maturation, CD8+ Cytotoxic T Lymphocyte (CTL) granule release and Antigen Presenting Cell (APC) function and maturation state.[13-15] Tregs mediate these functions mainly by 4 mechanisms including; 1) production of suppressive cytokines, 2) direct cytolytic activity, 3) cytokine (IL-2) deprivation, and 4) cell contact-induced cell modulation.[16,17]

Based on acquisition of CD25, Tregs can be divided into two subsets: natural Treg (nTreg) cells and adaptive or induced Treg (iTreg) cells. nTregs acquire expression of CD25 in thymus whereas iTregs acquire CD25 expression in the periphery. However, utility of CD25 as a marker of Tregs is limited because of its expression on activated T cells as well. iTregs are generated extra-thymically and IL-2 is essential for their generation both *in vivo* and *in vitro*. Tr1 and Th3 cells represent other subsets of suppressor T cells. Tr1 cells do not express FoxP3, but produce high level of immunosuppressive cytokine, IL-10-,[18,19] whereas Th3 cells produce TGF-β, which also has immunosuppressive role.[20] Phenotypically, it is difficult to differentiate nTregs from iTregs as both subsets have similar characteristics and suppressive function. Both Treg subsets express CD25, FoxP3, Glucocorticoid-induced TNF- receptor (GITR) and Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) whereas, nTregs exhibit a higher expression of Programmed cell death-1 (PD-1), Neuropilin 1 (Nrp-1) and Helios compared to iTregs.[21] It has been reported that nTregs are generated when there is a need to control inflammatory responses to autoantigens, whereas iTregs are generated in response to stimulation with foreign antigens such as intestinal flora and food allergens.[22] The features distinguishing nTregs from iTregs have been summarized in Table 1.

Alteration in the Frequency and Function of Tregs in T1D

Several groups have reported alterations in the frequency, function and phenotype of Tregs in patients with T1D. Subjects with T1D may harbor lower frequency of Tregs in the peripheral blood.[11,42-44] Ryba-Stanisławowska, et al. showed that patients with T1D had a decreased percentage of circulating CD4+CD25hi Tregs and elevated levels of serum IL-12 and IL-18 in comparison to their healthy controls.[45] However, a few studies have also reported no alteration in the frequency of the Tregs in peripheral blood of T1D subjects.[46,47] A recent study by Xufré, et al. reported that the frequency of peripheral CD4+CD25hi Treg cells are similar between T1D subjects and healthy controls.[47] However, the yield of sorted Treg cells was found to be significantly lower in T1D subjects than in controls. Again, upon comparison of Treg cell phenotype between the two groups, the only difference observed was the low expression of GITR in T1D subjects.[47] Zoka, et al. studied the expression of CD25 on CD4+FoxP3+ cells and reported that T1D subjects have higher proportion of CD25-/low cells among CD4+FoxP3+ Treg cells.[48] Willcox, et al. analyzed postmortem pancreatic samples from T1D subjects.[30] FoxP3+ Tregs were only found in islets from a single subject, suggesting that the lack of local Treg cells might be important in the pathogenesis of T1D.[49]

Besides numbers, many studies have reported that Tregs isolated from peripheral blood of the T1D patients are defective in suppressive function.[50-53] Ferraro, et al. showed that Tregs from peripheral blood of T1D subjects have normal suppressive activity but Tregs isolated from Pancreatic Lymph Nodes (PLN) of same subjects are functionally defective.[12] It has also been reported that Tregs are unstable in T1D subjects since they lose the expression of FoxP3 due to defect in IL-2R signalling.[53] Another

Characteristics	Natural Tregs	Induced Tregs
Site of induction	Thymus	Secondary lymphoid organs, inflamed tissues
Co stimulation requirement	CD27,[23] CD28,[24] CD40L[25]	CD28,[26] CTLA4[27]
Cytokines requirement	IL-2,[28,29]	TGF-β,[26] IL-2[30]
Specificity	Predominantly self-antigens[31-33]	intestinal flora and environmental, food allergens[22,34,35]
Common markers	CD25, FoxP3, GITR and CTLA4,CD127 low	CD25, FoxP3, GITR and CTLA4, CD127 low
Specific Markers	Higher expression of PD1,[36] neuropilin 1,[21,37] Helios[38,39] and CD73[36]	Dapl1,Igfbp4[27,36]
Methylation status of TSDR of FoxP3 promoter	Demethylated/low TSDR methylation[36,40]	Intermediate TSDR methylation[41]

Table 1: Characteristic features of natural and induced Tregs.

study showed that T1D subjects harbor substantial percentage of cells with transient or unstable expression of FoxP3. These exFoxP3 cells produce inflammatory cytokines, indicative of a high degree of plasticity in Treg phenotype.[41,54] It has also been reported that the Teff cell population in T1D subjects are resistant to suppression by Tregs.[55,56] Thus, it is still unclear whether Treg cells from T1D patients have intrinsic defective function or whether the responder T cells are resistant to suppression, warranting the need for additional studies. Moreover, studies on the role of Tregs in T1D were performed on peripheral blood rather than pancreas or PLN, therefore the defects in local Tregs are not well known.

Potential of Tregs in Treatment of T1D

There are many evidences which show that Tregs have the potential to prevent destruction of pancreatic islets, thereby protecting from T1D. Hence, strategies to increase Treg cell numbers and/or function are being explored as potential therapeutic approaches in treating T1D. Most of the treatment regimens to reverse diabetes in NOD mice worked *via* induction of Tregs or proliferation of Tregs.[57-62] Therapy of T1D subjects with Tregs has been shown to prolong survival of pancreatic islets.[63] At the same time, the knowledge on use of different type of Tregs for their clinical applications has increased tremendously. Today, several groups are engaged in exploring different Treg cell types, sources, induction procedures and experimental systems in pursuit of generation of highly efficacious and stable Tregs for immunotherapy of T1D.

Approaches used for *in vitro* Induction of Tregs

Clinical use of Tregs is hindered by their low frequency in peripheral blood.[64] Therefore several methods have been developed for induction and expansion of Tregs (Table 2), few of which have led to trials in T1D subjects with varying success rates (Table 3). Generation of iTregs from CD25- T cells *in vivo* is still not fully understood. However it has been established that it requires TCR stimulation, IL-2 and TGF-β both *in vitro* and *in vivo*. Supplementation of other compounds such as rapamycin and All Trans Retinoic Acid (ATRA)[25,65] increase the yield and purity of Tregs.[66] Addition of TGF-β induces transcription of FoxP3 by a mechanism that involves transcription factors STAT3 and NFAT at the FoxP3 gene enhancer element.[67,68] Tregs induced in the presence of rapamycin/TGF-β are more stable than ATRA/TGF-β iTregs.[66] However, upon re-stimulation the expression of FoxP3 decreases in both the iTregs; which in turn may lead to loss of suppressive activity.[66] nTregs expanded in presence of rapamycin maintain FoxP3 expression and are highly suppressive than IL-2 expanded nTregs. Tregs expanded with anti CD3 and anti CD28 infused into T1D subjects have been shown to prolong the survival of pancreatic islets.[63] Recently Lu, et al. reported that iTregs induced from CD39+ naive T cells demonstrated enhanced proliferative and suppressive ability.[69] However, expanded nTregs are shown to be superior to fresh nTregs since i*n vitro* expansion improves their *in vivo* regulation.[7]

Methods	Tregs specificity	Reference
IL-10	Antigen specific	71
Immature DC	Antigen specific	72,73
Anti-CD3,anti-CD28+TGF-β	Polyclonal	74
Peptide +Irradiated PBMCs	Antigen specific	75
Anti-CD3,anti-CD28+IL-2+TGF- β	Antigen specific	76,77
Plasmacytoid DCs	Antigen Specific	78
Glucocorticoid induced leucine zipper expressing (GILZ) expressing DCs	Antigen specific	79
Mature DC+antigen	Antigen specific	80
Anti-CD3 and autologous APC	Polyclonal	81
PBMCs+ mesenchymal stem cells	Polyclonal	82
Programmed death ligand 1 (PDL1) coated beads	Polyclonal	83
IL-2 + irradiated APC + peptide	Antigen specific	84
Lentivirus T cell receptor gene transfer in nTregs	Antigen specific	85
CD40 activated B cells+antigen	Antigen specific	86
IL-2+TGF-β+APC	Polyclonal	77
Delta like 1 ligand (notch signaling)+ memory CD4+T cells	Polyclonal	87
IL-2+TGF-β+All trans retinoic acid (ATRA)	Polyclonal	66
IL-2+TGF- β + rapamycin	Polyclonal	66
PBMCs + human amniotic fluid stem cells (fHASC)	Polyclonal	88
Lentiviral insulin (B,9-23) epitope expression in hepatocytes	Antigen specific	89

Table 2: Approaches used for in vitro induction of Tregs.

Trial	Results/Impact	References
Anti CD3 treatment	increased iTregs, preserved residual endogenous β-cell mass	90
Insulin B-chain in incomplete Freund's adjuvant (IFA)	Induction of Tregs	91
Vitamin D3	Increase in percentage of Tregs,no differences in fasting C-peptide levels	92
GAD-Alum	Preservation of residual insulin secretion and induction of antigen specific Tregs	93
Administration of *ex vivo* expanded Tregs in children	Increase in the percentage of Tregs in peripheral blood, preservation of β cells	94
Rapamycin/IL-2 combination therapy	Increase in Tregs with transient β cell dysfunction	95
Ex vivo expanded Tregs infusion in adults	Prolonged survival of pancreatic islets	63
Anti-thymocyte globulin/G-CSF	Relative preservation of Tregs and β cells	96
Low-dose IL-2	Expansion of Tregs	94
Oral insulin	Increase in Tregs, decrease in hypoglycemic events	97

Table 3: Immunotherapeutic approaches involving induction/use of Tregs in T1D subjects.

Antigen Specific Tregs are More Potent than Polyclonal Tregs

Harnessing Tregs is a promising approach for treating autoimmune disease. Administration of polyclonal Tregs may be associated with significant off-target effects, including a global immunosuppression that may compromise beneficial immune responses to infections and cancer cells. Therefore, the objective of research in recent times has shifted to antigen specific therapeutic approaches that can reverse the disease by selectively halting the harmful immune response without requiring lifelong immune suppression. Adoptive transfer studies suggest that antigen specificity is required by Tregs for trafficking and maintenance in inflammatory tissues such as the pancreas in T1D.[98,99] Antigen specific Tregs are required at less number and are more efficient than polyclonal Tregs in suppressing autoimmune diabetes.[85,100,101] Previous studies have shown that small number of *in vitro* expanded antigen specific Tregs are sufficient to reverse T1D in comparison to large numbers of polyclonal Tregs.[100] Antigen specific Tregs have been reported to exhibit a much lower threshold for activation and may be activated by a broad range of loosely-defined analogs of their cognate antigen; normally it is conceivable that the polyclonal Tregs may have received sufficient signaling within the pancreas to become suppressive.[102] Besides, the site specific mode of action, antigen specific Tregs have the ability to act as bystander suppressor locally in the organ under attack. It has also been shown in mice that antigen-specific Tregs treat autoimmunity without compromising antibacterial immune response.[103] However, isolation of sufficient number of antigen specific Tregs is a major challenge, particularly when sampling is limited to peripheral blood. Moreover, success in inducing antigen-specific tolerance has been hampered by the inability to identify peptides triggering the diabetogenic *versus* the regulatory response.

Generation of Antigen Specific Tregs

Several protocols have been established to induce antigen specific Tregs. Groux, et al. described induction of antigen specific Tregs by stimulating CD4+ T cells with antigen and IL-10 *in vitro*. This resulted in generation of antigen specific IL-10 producing Tr1 cells.[71] Immature Dendritic Cells (DCs) as well as plasmacytoid DCs exhibit regulatory functions.[78,104-106] Therefore, these DCs, have been used to induce antigen-specific CD4+ Tregs from CD4+CD25-T cells.[78,79] Walker, et al. used the mature DCs loaded with hemagglutinin (306-319, PKYVKQNTLKLAT) to generate Influenza hemagglutinin epitopes specific Tregs from CD4+CD25- T cells.[75] CD40 activated B cells are more potent than immature DCs for the induction of antigen specific Tregs.[86,107] Wenwei, et al. developed a method for expansion of alloantigen specific Tregs using CD40 activated B cells as APCs.[107] Alice, et al. generated the islet antigen specific Tregs from CD4+CD25- T cells by growing them in presence of GAD65 and IL-2 and observed that GAD65 derived epitope specific Tregs exhibit bystander suppression in the presence of antigen. In the suppression assay these epitope specific Tregs suppressed not only proliferation of GAD specific Teff cells but also of Tetanus Toxoid (TT) specific Teff cells in the presence of GAD. However, this bystander suppression was not observed in absence of GAD65 peptides or when TT was present alone.[84] Therefore these observations indicate that it might be possible to reverse autoimmune diabetes by small number of epitope specific Tregs rather than having Tregs specific for all the diabetes associated antigens. Brusko, et al. used lentiviral T Cell Receptor (TCR) gene transfer system to generate antigen specific Tregs from murine nTregs.[85] Tregs generated using this approach effectively blocked antigen-specific Teff cell activity. Also, DCs treated with glucocorticoids, upregulate Glucocorti-

coid-induced leucine zipper (GILZ). GILZ expressing DCs in the presence of IL-10 induce antigen specific CD25hi CTLA4+ Tregs.[79] Recently Akbarpour, et al. transferred an immunodominant insulin epitope (B9-23) expressing lentivirus vector in hepatocytes of NOD mice. The therapy induced insulin specific Tregs that inhibited immune cell infiltration in the pancreatic islets and halted diabetes development.[89] While induction of antigen specific Tregs is difficult, analysis of their characteristics is also technically challenging. Following induction, either *ex* or *in vivo*, antigen specific Tregs can be sorted using MHC class II tetramers loaded with peptide of interest.[84] Latency-associated peptide (LAP) and Glycoprotein A Repetitions Predominant (GARP) protein have also been reported as markers to identify human antigen-specific Tregs.[108]

Stability of Tregs

Clinical usage of Treg cells is hindered due to their instability. Tregs have been shown to lose FoxP3 expression under inflammatory environment.[54,109-113] Proinflammatory environment may abrogate the suppressive activity of Tregs[114-116] or cause Teff cells resistant to suppression.[117] There are certain reports that show that plasticity of Tregs might play important role in pathogenesis of autoimmune diseases. Indeed, increased frequency of IFN-γ+FoxP3+ cells has been reported in subjects with T1D.[41] Th17 cells originating from FoxP3+ T cells have shown to play a key role in the pathogenesis of autoimmune arthritis.[118] Stable Tregs can be distinguished from the unstable ones on the basis of epigenetic modifications in the CpG-rich Treg Specific Demethylated Region (TSDR) of the FoxP3 locus.[66] Demethylation of the TSDR region correlates with the stability of FoxP3 gene. Strong methylation in the TSDR of FoxP3 promoter may be associated with unstable phenotype of Tregs. Analyzing the demethylation status of the TSDR in the FoxP3 may aid in distinguishing the stable Tregs from unstable Tregs.[36]

Role of Colonic Tregs

The gut immune system plays an important role in autoimmune diabetes. One of the most influential environmental factors that influences gut immune system is the gut microbiota. The development of clinical diabetes is preceded by intestinal alterations such as an aberrant intestinal microbiota, a leaky intestinal mucosal barrier and an altered mucosal immune system.[119] Therefore, a hygiene hypothesis has been postulated which suggested a reduction in childhood exposure to infections leading to the accelerated development of T1D.[120] The gut microbiota shape the mucosal immune system by controlling many types of T cells including the colonic regulatory T cells (cTregs) which are a type of induced Tregs. It has been proposed that pathogenic microbes promote T1D development by enhancing self-reactive T cells,[119] while many microbial species such as Clostridia species has been shown to be potent inducers of cTregs. The gut microbiota modulates local immune system by acting on various immune cells including DCs. These lamina propria CD103+ CD11c+ DCs direct the antigens that cross the epithelial barrier to the Gut Associated Lymphoid Tissue (GALT), and enable the differentiation of naïve CD4+ T cells to cTregs *via* TGF-β and retinoic acid.[104,121] These Tregs control inflammation *via* anti-inflammatory agents such as IL-10 and TGF-β.[122] Studies in T1D subjects have reported low frequency of FoxP3+ Tregs and an impaired differentiation of FoxP3+ Treg cells by intestinal CD103+CD11c+DCs.[11] Due to the immunological connection between the GALT and the PLN, the immunological changes taking place in the gut are reflected in the pancreas. Thus the impaired generation of Tregs in the gut alters the Teff/Treg cell balance in PLN and islets thereby promoting Teff cell responses against pancreatic self-antigens.[123,124] This leads to failure of self-tolerance and development of autoimmunity.

Strategies to Induce/Increase Abundance of cTregs

cTregs play a critical role in limiting the intestinal inflammation. They are constitutively present in the intestinal mucosa as well as the GALT and thus maintain immune homeostasis. However the breakdown of gut immune system leads to many autoimmune diseases including T1D. Hence various strategies have been developed to generate cTregs.

Animal studies have shown that intestinal colonization with commensal bacteria activate and expand Treg cells, as well as de novo generate cTregs. The colonization of germ free mice with Altered Schaedler Flora (ASF) species resulted in the generation of Tregs in colonic lamina propria. These Tregs limited the proliferation of Th1 and Th17 cells.[125] Furthermore a defined cocktail of 17 strains of clostridium species within the cluster IV, XIVa and XVIII of Clostridia strains has been shown to trigger the expression of TGF-β in the intestinal epithelial cells, thereby promoting the accumulation of FoxP3+ Tregs.[126] Also Polysaccharide A (PSA) secreted by *Bacteroides fragilis* has been shown to act *via* TLR2 expressed on CD4+ T cells which enable their conversion to FoxP3+ T cells that produce IL-10.[94] The specific *Bifidobacterium* strains present in healthy microbiota provides protection against pathogens; accordingly the early administration of *Bifidobacterium infantis* to mice attenuated the severity of colitis by the induction of Tregs in the Mesenteric Lymph Nodes (MLN).[127] With encouraging reports, several groups have come up with probiotics, live beneficial microorganisms that when administered continuously can induce gut immunity. In an important study, oral administration of probiotic VSL#3 to NOD mice during the early stages of life showed a delay in the progression of diabetes. This prevention was associated with the generation of IL-10 producing Tregs in the GALT.[128] Autoantigen specific therapies also hold great promise in the reversal of T1D by induction of oral tolerance. One such approach involved the administration of *Lactococcus lactis* for controlled secretion of GAD65 and IL10 in the gut, which favored the induction of Tregs.[129]

Besides bacteria, their metabolic products such as,

Short Chain Fatty Acids (SCFA) have been shown to affect the colonic health as they can penetrate the intestinal epithelium and restore intestinal immune responses. The administration of SCFA such as acetate, propionate and butyrate enabled the restoration of cTregs in germ free mice and significantly increased the expression of IL-10 and TGF-β in cTregs.[130] Among the SCFA, butyrate has received a lot of attention due to its effect on colonic function. The dietary administration of butyrylated high amylose maize starches to mice showed an increase in the frequency of cTregs.[131] Butyrate is also well known to epigenetically modify the FoxP3 gene by inhibiting the class I and IIa of histone deacetylases, thereby increasing the FoxP3 expression and differentiation of Treg cells.[132-134] The colonic DCs and macrophages express the cell surface receptor Gpr109a.[135,136] Butyrate acts *via* these receptors and induces the expression of anti-inflammatory molecules such as IL-10 and aldehyde dehydrogenase (Aldh1a), thereby supporting the differentiation of cTregs.[137,138] Additionally, intervention strategies such as dietary supplementation with 1,2 dihydroxy-vitamin D (1,25(OH)2D3), an active form of vitamin D promotes the development of FoxP3+ Treg cells and inhibits the differentiation of Th1 and Th17 cells.[139] High doses of vitamin D3 safely reduced diabetes development by preventing insulitis and preserving β cell mass in NOD mice.[140] Also the deficiency of Vitamin B9 or folic acid derived from diet and commensal bacteria showed marked reduction in gut FoxP3+ Treg cells.[141] In addition to induction approaches, homing of T lymphocytes to the gut is also important in induction of cTregs and impaired homing of T cells is implicated in many inflammatory diseases. For example, GPR15, an orphan heterotrimeric guanine nucleotide-binding protein (G protein) coupled receptor, controls the specific homing of FoxP3 Tregs, to the large intestine lamina propria, and its expression can be modulated by gut microbiota and TGF-β.[142]

Despite the difficulties in characterization of induced Tregs, there is an increasing awareness about the importance of induction of immune tolerance *via* gut and generation of cTregs have come to the forefront as an actively pursued area of research, in prevention of autoimmune diseases like T1D.

CONCLUSIONS

Many immunotherapeutic approaches including self-antigens and immune modulating agents have been tried to tackle autoimmunity observed in T1D. Most of these treatment strategies have failed to prevent or improve the clinical outcome of the disease. There are multiple etiologies that are known to cause β cell destruction in T1D. Hence targeting a single factor may not provide a lifelong preservation of the β cell mass in T1D. While the defects in number and function of Tregs in T1D were known long ago, research on application of Tregs in T1D has picked up more in recent years. Today there are several choices available in immunotherapeutic approaches with Tregs, ranging from their type (natural *versus* induced), source (peripheral *versus* colonic) or specificity (polyclonal *versus* antigen-specific) or methods of induction (direct *versus* indirect) and expansion (*in-vitro versus in-vivo*), each of which has its specific advantages and limitations. Regardless of the variety, Tregs have opened up new vistas in treatment of T1D. With growing understanding about the generation of different types of Tregs and their clinical applications, the use of Tregs in future treatment of T1D looks quite promising. We believe, Tregs might provide benefit in the form of a combination therapy that attenuates autoimmunity towards the pancreas, ultimately preserving β cell mass.

CONFLICTS OF INTEREST: None.

REFERENCES

1. Knight RR, Kronenberg D, Zhao M, et al. Human β-cell killing by autoreactive preproinsulin-specific CD8 T cells is predominantly granule-mediated with the potency dependent upon T-cell receptor avidity. *Diabetes*. 2013; 62(1): 205-213. doi: 10.2337/db12-0315

2. Luce S, Lemonnier F, Briand J-P, et al. Single insulin-specific CD8+ T cells show characteristic gene expression profiles in human type 1 diabetes. *Diabetes*. 2011; 60(12): 3289-3299. doi: 10.2337/db11-0270

3. Skowera A, Ladell K, McLaren JE, et al. β-cell-specific CD8 T cell phenotype in type 1 diabetes reflects chronic autoantigen exposure. *Diabetes*. 2014; DB-140332.

4. Sachdeva N, Paul M, Badal D, et al. Preproinsulin specific CD8+ T cells in subjects with latent autoimmune diabetes show lower frequency and different pathophysiological characteristics than those with type 1 diabetes. *Clinical Immunology*. 2015. doi: 10.1016/j.clim.2015.01.005

5. Sakaguchi S. Naturally arising Foxp3-expressing CD25+ CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nature immunology.* 2005; 6(4): 345-352. doi: 10.1038/ni1178

6. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Pillars article: immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J. Immunol*. 1995.

7. Maloy KJ, Powrie F. Regulatory T cells in the control of immune pathology. *Nature immunology*. 2001; 2(9): 816-822. doi: 10.1038/ni0901-816

8. Shevach EM. CD4+ CD25+ suppressor T cells: more questions than answers. *Nature Reviews Immunology.* 2002; 2(6): 389-400. doi: 10.1038/nri821

9. Ramsdell F. Foxp3 and natural regulatory T cells: key to a cell

lineage? *Immunity*. 2003; 19(2): 165-168.

10. Liu W, Putnam AL, Xu-Yu Z, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. *The Journal of experimental medicine*. 2006; 203(7): 1701-1711. doi: 10.1084/jem.20060772

11. Badami E, Sorini C, Coccia M, et al. Defective differentiation of regulatory FoxP3+ T cells by small-intestinal dendritic cells in patients with type 1 diabetes. *Diabetes*. 2011; 60(8): 2120-2124. doi: 10.2337/db10-1201

12. Ferraro A, Socci C, Stabilini A, et al. Expansion of Th17 cells and functional defects in T regulatory cells are key features of the pancreatic lymph nodes in patients with type 1 diabetes. *Diabetes*. 2011; 60(11): 2903-2913. doi: 10.2337/db11-0090

13. Taams LS, Boot EP, van Eden W, Wauben MH. Anergic T cells modulate the T-cell activating capacity of antigen-presenting cells. *Journal of autoimmunity*. 2000; 14(4): 335-341.

14. Eddahri F, Oldenhove G, Denanglaire S, Urbain J, Leo O, Andris F. CD4+ CD25+ regulatory T cells control the magnitude of T-dependent humoral immune responses to exogenous antigens. *European journal of immunology*. 2006; 36(4): 855-863. doi: 10.1002/eji.200535500

15. Mempel TR, Pittet MJ, Khazaie K, et al. Regulatory T cells reversibly suppress cytotoxic T cell function independent of effector differentiation. *Immunity*. 2006; 25(1): 129-141.

16. Bluestone JA, Tang Q. How do CD4+ CD25+ regulatory T cells control autoimmunity? *Current opinion in immunology*. 2005; 17(6): 638-642.

17. Tang Q, Bluestone JA. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation. *Nature immunology*. 2008; 9(3): 239-244. doi: 10.1038/ni1572

18. Roncarolo MG, Bacchetta R, Bordignon C, Narula S, Levings MK. Type 1 T regulatory cells. I*mmunological reviews*. 2001; 182(1): 68-79. doi: 10.1034/j.1600-065X.2001.1820105.x

19. Pot C, Apetoh L, Kuchroo VK, editors. Type 1 regulatory T cells (Tr1) in autoimmunity. *Seminars in immunology*. Elsevier, 2011. doi: 10.1016/j.smim.2011.07.005

20. Okamura T, Fujio K, Sumitomo S, Yamamoto K. Roles of LAG3 and EGR2 in regulatory T cells. *Annals of the rheumatic diseases*. 2012; 71(Suppl 2): i96-i100. doi: 10.1136/annrheumdis-2011-200588

21. Yadav M, Louvet C, Davini D, et al. Neuropilin-1 distinguishes natural and inducible regulatory T cells among regulatory T cell subsets *in vivo*. *The Journal of experimental medicine*. 2012; 209(10): 1713-1722. doi: 10.1084/jem.20120822

22. de Lafaille MAC, Lafaille JJ. Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor? *Immunity*. 2009; 30(5): 626-635. doi: 10.1016/j.immuni.2009.05.002

23. Lu L, Ma J, Li Z, et al. All-trans retinoic acid promotes TGF-β-induced Tregs via histone modification but not DNA demethylation on Foxp3 gene locus. *PloS one*. 2011; 6(9): e24590. doi: 10.1371/journal.pone.0024590

24. Tai X, Cowan M, Feigenbaum L, Singer A. CD28 costimulation of developing thymocytes induces Foxp3 expression and regulatory T cell differentiation independently of interleukin 2. *Nature immunology*. 2005; 6(2): 152-162. doi: 10.1038/ni1160

25. Benson MJ, Pino-Lagos K, Rosemblatt M, Noelle RJ. All-trans retinoic acid mediates enhanced T reg cell growth, differentiation, and gut homing in the face of high levels of co-stimulation. *The Journal of experimental medicine*. 2007; 204(8): 1765-1774. doi: 10.1084/jem.20070719

26. Shevach EM, Tran DQ, Davidson TS, Andersson J. The critical contribution of TGF-β to the induction of Foxp3 expression and regulatory T cell function. *European journal of immunology*. 2008; 38(4): 915-917. doi: 10.1002/eji.200738111

27. Bilate AM, Lafaille JJ. Induced CD4+ Foxp3+ regulatory T cells in immune tolerance. *Annual review of immunology*. 2012; 30: 733-758. doi: 10.1146/annurev-immunol-020711-075043

28. Salomon B, Lenschow DJ, Rhee L, et al. B7/CD28 costimulation is essential for the homeostasis of the CD4+ CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity*. 2000; 12(4): 431-440.

29. Cheng G, Yu A, Dee MJ, Malek TR. IL-2R signaling is essential for functional maturation of regulatory T cells during thymic development. *The Journal of Immunology*. 2013; 190(4): 1567-1575. doi: 10.4049/jimmunol.1201218

30. Stritesky GL, Jameson SC, Hogquist KA. Selection of self-reactive T cells in the thymus. *Annual review of immunology*. 2012; 30: 95.

31. Hsieh C-S, Lee H-M, Lio C-WJ. Selection of regulatory T cells in the thymus. *Nature Reviews Immunology*. 2012; 12(3): 157-167. doi: 10.1038/nri3155

32. Josefowicz SZ, Lu L-F, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. *Annual review of immunology*. 2012; 30: 531-564. doi: 10.1146/annurev.immunol.25.022106.141623

33. Lee H-M, Bautista JL, Scott-Browne J, Mohan JF, Hsieh

C-S. A broad range of self-reactivity drives thymic regulatory T cell selection to limit responses to self. *Immunity*. 2012; 37(3): 475-486. doi: 10.1016/j.immuni.2012.07.009

34. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal bacterium of the intestinal microbiota. *Proceedings of the National Academy of Sciences*. 2010; 107(27): 12204-12209. doi: 10.1073/pnas.0909122107

35. Lathrop SK, Bloom SM, Rao SM, et al. Peripheral education of the immune system by colonic commensal microbiota. *Nature*. 2011; 478(7368): 250-254. doi: 10.1038/nature10434

36. Lin X, Chen M, Liu Y, et al. Advances in distinguishing natural from induced Foxp3+ regulatory T cells. *International journal of clinical and experimental pathology*. 2013; 6(2): 116.

37. Bruder D, Probst-Kepper M, Westendorf AM, et al. Frontline: Neuropilin-1: a surface marker of regulatory T cells. *European journal of immunology*. 2004; 34(3): 623-630. doi: 10.1002/eji.200324799

38. Thornton AM, Korty PE, Tran DQ, et al. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. *The Journal of Immunology*. 2010; 184(7): 3433-3441. doi: 10.4049/jimmunol.0904028

39. Kim YC, Bhairavabhotla R, Yoon J, et al. Oligodeoxynucleotides stabilize Helios-expressing Foxp3+ human T regulatory cells during in vitro expansion. *Blood*. 2012; 119(12): 2810-2818. doi: 10.1182/blood-2011-09-377895

40. Lal G, Bromberg JS. Epigenetic mechanisms of regulation of Foxp3 expression. *Blood*. 2009; 114(18): 3727-3735. doi: 10.1182/blood-2009-05-219584

41. McClymont SA, Putnam AL, Lee MR, et al. Plasticity of human regulatory T cells in healthy subjects and patients with type 1 diabetes. *The Journal of Immunology*. 2011; 186(7): 3918-3926. doi: 10.4049/jimmunol.1003099

42. Kukreja A, Cost G, Marker J, et al. Multiple immuno-regulatory defects in type-1 diabetes. *The Journal of clinical investigation*. 2002; 109(109 (1)): 131-140. doi: 10.1172/JCI13605

43. Ryba M, Rybarczyk-Kapturska K, Zorena K, Myśliwiec M, Myśliwska J. Lower Frequency of CD62L high and Higher Frequency of TNFR2. *Mediators of inflammation*. 2011; 2011. doi: 10.1155/2011/645643

44. Ryba-Stanisławowska M, Skrzypkowska M, Myśliwska J, Myśliwiec M. The serum IL-6 profile and Treg/Th17 peripheral cell populations in patients with type 1 diabetes. *Mediators of inflammation*. 2013; 2013. doi: 10.1155/2013/205284

45. Ryba-Stanisławowska M, Rybarczyk-Kapturska K, Myśliwiec M, Myśliwska J. Elevated Levels of Serum IL-12 and IL-18 are Associated with Lower Frequencies of CD4+ CD25highFOXP3+ Regulatory T cells in Young Patients with Type 1 Diabetes. *Inflammation*. 2014; 37(5): 1513-1520. doi: 10.1007/s10753-014-9878-1

46. Brusko T, Wasserfall C, McGrail K, et al. No alterations in the frequency of FOXP3+ regulatory T-cells in type 1 diabetes. *Diabetes*. 2007; 56(3): 604-612. doi: 10.2337/db06-1248

47. Xufré C, Costa M, Roura-Mir C, et al. Low frequency of GITR+ T cells in ex vivo and in vitro expanded Treg cells from type 1 diabetic patients. *International immunology*. 2013: dxt020.

48. Zóka A, Barna G, Somogyi A, et al. Extension of the CD4+ Foxp3+ CD25-/low regulatory T-cell subpopulation in type 1 diabetes mellitus. *Autoimmunity*. 2014; 1-9. doi: 10.3109/08916934.2014.992518

49. Willcox A, Richardson S, Bone A, Foulis A, Morgan N. Analysis of islet inflammation in human type 1 diabetes. *Clinical & Experimental Immunology*. 2009; 155(2): 173-181. doi: 10.1111/j.1365-2249.2008.03860.x

50. Lindley S, Dayan CM, Bishop A, Roep BO, Peakman M, Tree TI. Defective suppressor function in CD4+ CD25+ T-cells from patients with type 1 diabetes. *Diabetes*. 2005; 54(1): 92-99. doi: 10.2337/diabetes.54.1.92

51. Brusko TM, Wasserfall CH, Clare-Salzler MJ, Schatz DA, Atkinson MA. Functional defects and the influence of age on the frequency of CD4+ CD25+ T-cells in type 1 diabetes. *Diabetes*. 2005; 54(5): 1407-1414. doi: 10.2337/diabetes.54.5.1407

52. Haseda F, Imagawa A, Murase-Mishiba Y, Terasaki J, Hanafusa T. CD4+ CD45RA− FoxP3high activated regulatory T cells are functionally impaired and related to residual insulin-secreting capacity in patients with type 1 diabetes. *Clinical & Experimental Immunology*. 2013; 173(2): 207-216. doi: 10.1111/cei.12116

53. Long SA, Cerosaletti K, Bollyky PL, et al. Defects in IL-2R signaling contribute to diminished maintenance of FOXP3 expression in CD4+ CD25+ regulatory T-cells of type 1 diabetic subjects. *Diabetes*. 2010; 59(2): 407-415. doi: 10.2337/db09-0694

54. Zhou X, Bailey-Bucktrout SL, Jeker LT, et al. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. *Nature immunology*. 2009; 10(9): 1000-1007. doi: 10.1038/ni.1774

55. Schneider A, Rieck M, Sanda S, Pihoker C, Greenbaum C,

Buckner JH. The effector T cells of diabetic subjects are resistant to regulation via CD4+ FOXP3+ regulatory T cells. *The Journal of Immunology*. 2008; 181(10): 7350-7355. doi: 10.4049/jimmunol.181.10.7350

56. Lawson J, Tremble J, Dayan C, et al. Increased resistance to CD4+ CD25hi regulatory T cell-mediated suppression in patients with type 1 diabetes. *Clinical & Experimental Immunology*. 2008; 154(3): 353-359. doi: 10.1111/j.1365-2249.2008.03810.x

57. Zhang J, Gao W, Yang X, et al. Tolerogenic vaccination reduced effector memory CD4 T cells and induced effector memory Treg cells for type I diabetes treatment. *PloS one*. 2013; 8(7): e70056. doi: 10.1371/journal.pone.0070056

58. Johnson MC, Garland AL, Nicolson SC, et al. β-Cell–Specific IL-2 Therapy Increases Islet Foxp3+ Treg and Suppresses Type 1 Diabetes in NOD Mice. *Diabetes*. 2013; 62(11): 3775-3784. doi: 10.2337/db13-0669

59. Bilbao D, Luciani L, Johannesson B, Piszczek A, Rosenthal N. Insulin-like growth factor-1 stimulates regulatory T cells and suppresses autoimmune disease. *EMBO molecular medicine*. 2014; e201303376.

60. Tian J, Dang H, Nguyen AV, Chen Z, Kaufman DL. Combined therapy with GABA and proinsulin/alum acts synergistically to restore long-term normoglycemia by modulating T-Cell autoimmunity and promoting β-Cell replication in newly diabetic NOD mice. *Diabetes*. 2014; 63(9): 3128-3134. doi: 10.2337/db13-1385

61. Turner MS, Isse K, Fischer DK, Turnquist HR, Morel PA. Low TCR signal strength induces combined expansion of Th2 and regulatory T cell populations that protect mice from the development of type 1 diabetes. *Diabetologia*. 2014; 57(7): 1428-1436. doi: 10.1007/s00125-014-3233-9

62. Lin G-J, Sytwu H-K, Yu J-C, et al. Dimethyl sulfoxide inhibits spontaneous diabetes and autoimmune recurrence in non-obese diabetic mice by inducing differentiation of regulatory T cells. *Toxicology and applied pharmacology*. 2015; 282(2): 207-214. doi: 10.1016/j.taap.2014.11.012

63. Marek-Trzonkowska N, Myśliwiec M, Dobyszuk A, et al. Therapy of type 1 diabetes with CD4+ CD25 high CD127-regulatory T cells prolongs survival of pancreatic islets-Results of one year follow-up. *Clinical Immunology*. 2014; 153(1): 23-30. doi: 10.1016/j.clim.2014.03.016

64. Bluestone JA. Regulatory T-cell therapy: is it ready for the clinic? *Nature Reviews Immunology*. 2005; 5(4): 343-349. doi: 10.1038/nri1574

65. Hill JA, Hall JA, Sun C-M, et al. Retinoic acid enhances Foxp3 induction indirectly by relieving inhibition from CD4+ CD44 hi cells. *Immunity*. 2008; 29(5): 758-770. doi: 10.1016/j.immuni.2008.09.018

66. Rossetti M, Spreafico R, Saidin S, et al. *Ex vivo*-expanded but not *in vitro*-induced human regulatory T Cells are candidates for cell therapy in autoimmune diseases thanks to stable demethylation of the FOXP3 regulatory T Cell-specific demethylated region. *The Journal of Immunology*. 2015; 194(1): 113-124. doi: 10.4049/jimmunol.1401145

67. Fantini M, Becker C, Monteleone G, Pallone F, Galle P, Neurath M. TGF-beta induces a regulatory phenotype in CD4+ CD25-T cells through FoxP3 induction and dowuregulation of Smad7. Gastroenterology. Wb Saunders Co Independence Square West Curtis Center, Ste 300, Philadelphia, PA 19106-3399 USA, 2004 .

68. Josefowicz SZ, Rudensky A. Control of regulatory T cell lineage commitment and maintenance. *Immunity*. 2009; 30(5): 616-625. doi: 10.1016/j.immuni.2009.04.009

69. Lu Y, Gu J, Lu H, et al. iTreg induced from CD39+ naive T cells demonstrate enhanced proliferate and suppressive ability. *International immunopharmacology*. 2015. doi: 10.1016/j.intimp.2015.03.039

70. Chai J-G, Coe D, Chen D, Simpson E, Dyson J, Scott D. In vitro expansion improves in vivo regulation by CD4+ CD25+ regulatory T cells. *The Journal of Immunology*. 2008; 180(2): 858-869. doi: 10.4049/jimmunol.180.2.858

71. Groux H, O'Garra A, Bigler M, et al. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature*. 1997; 389(6652): 737-742.

72. Jonuleit H, Schmitt E, Schuler G, Knop J, Enk AH. Induction of interleukin 10–producing, nonproliferating CD4+ T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *The Journal of experimental medicine*. 2000; 192(9): 1213-1222.

73. Gad M, Kristensen NN, Kury E, Claesson MH. Characterization of T-regulatory cells, induced by immature dendritic cells, which inhibit enteroantigen-reactive colitis-inducing T-cell responses *in vitro* and *in vivo*. *Immunology*. 2004; 113(4): 499-508. doi: 10.1111/j.1365-2567.2004.01977.x

74. Rao PE, Petrone AL, Ponath PD. Differentiation and expansion of T cells with regulatory function from human peripheral lymphocytes by stimulation in the presence of TGF-β. *The Journal of Immunology*. 2005; 174(3): 1446-1455. doi: 10.4049/jimmunol.174.3.1446

75. Walker MR, Carson BD, Nepom GT, Ziegler SF, Buckner JH. De novo generation of antigen-specific CD4+ CD25+ regulatory T cells from human CD4+ CD25–cells. *Proceedings of the*

National Academy of Sciences of the United States of America. 2005; 102(11): 4103-4108.

76. DiPaolo RJ, Brinster C, Davidson TS, Andersson J, Glass D, Shevach EM. Autoantigen-specific TGFβ-induced Foxp3+ regulatory T cells prevent autoimmunity by inhibiting dendritic cells from activating autoreactive T cells. *The Journal of Immunology*. 2007; 179(7): 4685-4693. doi: 10.4049/jimmunol.179.7.4685

77. Zhao C, Shi G, Vistica BP, et al. Induced regulatory T-cells (iTregs) generated by activation with anti-CD3/CD28 antibodies differ from those generated by the physiological-like activation with antigen/APC. *Cellular immunology*. 2014; 290(2): 179-184. doi: 10.1016/j.cellimm.2014.06.004

78. Kang H-K, Liu M, Datta SK. Low-dose peptide tolerance therapy of lupus generates plasmacytoid dendritic cells that cause expansion of autoantigen-specific regulatory T cells and contraction of inflammatory Th17 cells. *The Journal of Immunology*. 2007; 178(12): 7849-7858. doi: 10.4049/jimmunol.178.12.7849

79. Hamdi H, Godot V, Maillot M-C, et al. Induction of antigen-specific regulatory T lymphocytes by human dendritic cells expressing the glucocorticoid-induced leucine zipper. *Blood*. 2007; 110(1): 211-219.

80. Tarbell KV, Petit L, Zuo X, et al. Dendritic cell–expanded, islet-specific CD4+ CD25+ CD62L+ regulatory T cells restore normoglycemia in diabetic NOD mice. *The Journal of experimental medicine*. 2007; 204(1): 191-201. doi: 10.1084/jem.20061631

81. Pillai V, Ortega SB, Wang C, Karandikar NJ. Transient regulatory T-cells: a state attained by all activated human T-cells. *Clinical immunology*. 2007; 123(1): 18-29. doi: 10.1016/j.clim.2006.10.014

82. Prevosto C, Zancolli M, Canevali P, Zocchi MR, Poggi A. Generation of CD4+ or CD8+ regulatory T cells upon mesenchymal stem cell-lymphocyte interaction. *Haematologica*. 2007; 92(7): 881-888. doi: 10.3324/haematol.11240

83. Francisco LM, Salinas VH, Brown KE, et al. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *The Journal of experimental medicine*. 2009; 206(13): 3015-3029. doi: 10.1084/jem.20090847

84. Long SA, Walker MR, Rieck M, et al. Functional islet-specific Treg can be generated from CD4+ CD25- T cells of healthy and type 1 diabetic subjects. *European journal of immunology*. 2009; 39(2): 612-620. doi: 10.1002/eji.200838819

85. Brusko TM, Koya RC, Zhu S, et al. Human antigen-specific regulatory T cells generated by T cell receptor gene transfer. *PloS one*. 2010; 5(7): e11726. doi: 10.1371/journal.pone.0011726

86. Zheng J, Liu Y, Lau Y-L, Tu W. CD40-activated B cells are more potent than immature dendritic cells to induce and expand CD4+ regulatory T cells. *Cellular & molecular immunology*. 2010; 7(1): 44-50.

87. Mota C, Nunes-Silva V, Pires AR, et al. Delta-like 1-mediated notch signaling enhances the *in vitro* conversion of human memory CD4 T Cells into FOXP3-expressing regulatory T Cells. *The Journal of Immunology*. 2014; 193(12): 5854-5862. doi: 10.4049/jimmunol.1400198

88. Romani R, Pirisinu I, Calvitti M, et al. Stem cells from human amniotic fluid exert immunoregulatory function via secreted indoleamine 2, 3-dioxygenase1. *Journal of cellular and molecular medicine*. 2015. doi: 10.1111/jcmm.12534

89. Akbarpour M, Goudy KS, Cantore A, et al. Insulin B chain 9-23 gene transfer to hepatocytes protects from type 1 diabetes by inducing Ag-specific FoxP3+ Tregs. *Science Translational Medicine*. 2015; 7(289): 289ra81-289ra81. doi: 10.1126/scitranslmed.aaa3032

90. Chatenoud L, Bluestone JA. CD3-specific antibodies: a portal to the treatment of autoimmunity. *Nature Reviews Immunology*. 2007; 7(8): 622-632. doi: 10.1038/nri2134

91. Orban T, Farkas K, Jalahej H, et al. Autoantigen-specific regulatory T cells induced in patients with type 1 diabetes mellitus by insulin B-chain immunotherapy. *Journal of autoimmunity*. 2010; 34(4): 408-415. doi: 10.1016/j.jaut.2009.10.005

92. Bock G, Prietl B, Mader JK, et al. The effect of vitamin D supplementation on peripheral regulatory T cells and β cell function in healthy humans: a randomized controlled trial. *Diabetes/metabolism research and reviews*. 2011; 27(8): 942-945. doi: 10.1002/dmrr.1276

93. Hjorth M, Axelsson S, Rydén A, Faresjö M, Ludvigsson J, Casas R. GAD-alum treatment induces GAD 65-specific CD4+ CD25 high FOXP3+ cells in type 1 diabetic patients. *Clinical immunology*. 2011; 138(1): 117-126.

94. Marek-Trzonkowska N, Myśliwiec M, Dobyszuk A, et al. Administration of CD4+ CD25 high CD127-regulatory T Cells preserves β-Cell function in Type 1 Diabetes in children. *Diabetes care*. 2012; 35(9): 1817-1820. doi: 10.2337/dc12-0038

95. Long SA, Rieck M, Sanda S, et al. Rapamycin/IL-2 combination therapy in patients with type 1 diabetes augments Tregs yet transiently impairs β-cell function. *Diabetes*. 2012; 61(9): 2340-2348. doi: 10.2337/db12-0049

96. Haller MJ, Gitelman SE, Gottlieb PA, et al. Anti-thymocyte globulin/G-CSF treatment preserves β cell function in patients with established type 1 diabetes. *The Journal of clinical investigation*. 2014; 125(125(1)).

97. Bonifacio E, Ziegler A-G, Klingensmith G, et al. Effects of high-dose oral insulin on immune responses in children at high risk for type 1 diabetes: the Pre-POINT randomized clinical trial. *JAMA*. 2015; 313(15): 1541-1549. doi: 10.1001/jama.2015.2928

98. Penaranda C, Bluestone JA. Is antigen specificity of autoreactive T cells the key to islet entry? *Immunity*. 2009; 31(4): 534-536. doi: 10.1016/j.immuni.2009.09.006

99. Lennon GP, Bettini M, Burton AR, et al. T cell islet accumulation in type 1 diabetes is a tightly regulated, cell-autonomous event. *Immunity*. 2009; 31(4): 643-653. doi: 10.1016/j.immuni.2009.07.008

100. Tang Q, Henriksen KJ, Bi M, et al. *In vitro*-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *The Journal of experimental medicine*. 2004; 199(11): 1455-1465. doi: 10.1084/jem.20040139

101. Bluestone JA, Tang Q. Therapeutic vaccination using CD4+ CD25+ antigen-specific regulatory T cells. *Proceedings of the National Academy of Sciences*. 2004; 101(Suppl 2): 14622-14626. doi: 10.1073/pnas.0405234101

102. Larkin J, Picca CC, Caton AJ. Activation of CD4+ CD25+ regulatory T cell suppressor function by analogs of the selecting peptide. *European journal of immunology*. 2007; 37(1): 139-146. doi: 10.1002/eji.200636577

103. Kasagi S, Zhang P, Che L, et al. In vivo–generated antigen-specific regulatory T cells treat autoimmunity without compromising antibacterial immune response. *Science translational medicine*. 2014; 6(241): 241ra78-241ra78. doi: 10.1126/scitranslmed.3008895

104. Coombes JL, Siddiqui KR, Arancibia-Cárcamo CV, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-β–and retinoic acid–dependent mechanism. *The Journal of experimental medicine*. 2007; 204(8): 1757-1764. doi: 10.1084/jem.20070590

105. Ito T, Yang M, Wang Y-H, et al. Plasmacytoid dendritic cells prime IL-10–producing T regulatory cells by inducible costimulator ligand. *The Journal of experimental medicine*. 2007; 204(1): 105-115. doi: 10.1084/jem.20061660

106. Yamazaki S, Dudziak D, Heidkamp GF, et al. CD8+ CD205+ splenic dendritic cells are specialized to induce Foxp3+ regulatory T cells. *The Journal of Immunology*. 2008; 181(10): 6923-6933. doi: 10.4049/jimmunol.181.10.6923

107. Tu W, Lau Y-L, Zheng J, et al. Efficient generation of human alloantigen-specific CD4+ regulatory T cells from naive precursors by CD40-activated B cells. *Blood*. 2008; 112(6): 2554-2562. doi: 10.1182/blood-2008-04-152041

108. Noyan F, Lee YS, Zimmermann K, et al. Isolation of human antigen-specific regulatory T cells with high suppressive function. *European journal of immunology*. 2014; 44(9): 2592-2602. doi: 10.1002/eji.201344381

109. Yang XO, Nurieva R, Martinez GJ, et al. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. *Immunity*. 2008; 29(1): 44-56. doi: 10.1016/j.immuni.2008.05.007

110. Komatsu N, Mariotti-Ferrandiz ME, Wang Y, Malissen B, Waldmann H, Hori S. Heterogeneity of natural Foxp3+ T cells: a committed regulatory T-cell lineage and an uncommitted minor population retaining plasticity. *Proceedings of the National Academy of Sciences*. 2009; 106(6): 1903-1908. doi: 10.1073/pnas.0811556106

111. Oldenhove G, Bouladoux N, Wohlfert EA, et al. Decrease of Foxp3+ Treg cell number and acquisition of effector cell phenotype during lethal infection. *Immunity*. 2009; 31(5): 772-786. doi: 10.1016/j.immuni.2009.10.001

112. Miyao T, Floess S, Setoguchi R, et al. Plasticity of Foxp3+ T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. *Immunity*. 2012; 36(2): 262-275. doi: 10.1016/j.immuni.2011.12.012

113. Yurchenko E, Shio MT, Huang TC, et al. Inflammation-driven reprogramming of CD4+ Foxp3+ regulatory T cells into pathogenic Th1/Th17 T effectors is abrogated by mTOR inhibition *in vivo*. *PloS one*. 2012; 7(4): e35572. doi: 10.1371/journal.pone.0035572

114. Pasare C, Medzhitov R. Toll pathway-dependent blockade of CD4+ CD25+ T cell-mediated suppression by dendritic cells. *Science*. 2003; 299(5609): 1033-1036. doi: 10.1126/science.1078231

115. Valencia X, Stephens G, Goldbach-Mansky R, Wilson M, Shevach EM, Lipsky PE. TNF downmodulates the function of human CD4+ CD25hi T-regulatory cells. *Blood*. 2006; 108(1): 253-261. doi: 10.1182/blood-2005-11-4567

116. Peluso I, Fantini MC, Fina D, et al. IL-21 counteracts the regulatory T cell-mediated suppression of human CD4+ T lymphocytes. *The Journal of Immunology*. 2007; 178(2): 732-739. doi: 10.4049/jimmunol.178.2.732

117. Ruprecht CR, Gattorno M, Ferlito F, et al. Coexpression of CD25 and CD27 identifies FoxP3+ regulatory T cells in inflamed synovia. *The Journal of experimental medicine*. 2005; 201(11): 1793-1803. doi: 10.1084/jem.20050085

118. Komatsu N, Okamoto K, Sawa S, et al. Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis. *Nature medicine*. 2014; 20(1): 62-68. doi: 10.1038/nm.3432

119. Vaarala O, Atkinson MA, Neu J. The Perfect storm for type 1 diabetes the complex interplay between intestinal microbiota, gut permeability, and mucosal immunity. *Diabetes*. 2008; 57(10): 2555-2562. doi: 10.2337/db08-0331

120. Kukreja A, Maclaren NK. NKT cells and type-1 diabetes and the" hygiene hypothesis" to explain the rising incidence rates. *Diabetes technology & therapeutics*. 2002; 4(3): 323-333. doi: 10.1089/152091502760098465

121. Sun C-M, Hall JA, Blank RB, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *The Journal of experimental medicine*. 2007; 204(8): 1775-1785. doi: 10.1084/jem.20070602

122. Coombes JL, Powrie F. Dendritic cells in intestinal immune regulation. *Nature Reviews Immunology*. 2008; 8(6): 435-446. doi: 10.1038/nri2335

123. Jaakkola I, Jalkanen S, Hänninen A. Diabetogenic T cells are primed both in pancreatic and gut-associated lymph nodes in NOD mice. *European journal of immunology*. 2003; 33(12): 3255-3264. doi: 10.1002/eji.200324405

124. Hänninen A, Nurmela R, Maksimow M, Heino J, Jalkanen S, Kurts C. Islet β-cell-specific T cells can use different homing mechanisms to infiltrate and destroy pancreatic islets. *The American journal of pathology*. 2007; 170(1): 240-250. doi: 10.2353/ajpath.2007.060142

125. Geuking MB, Cahenzli J, Lawson MA, et al. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity*. 2011; 34(5): 794-806. doi: 10.1016/j.immuni.2011.03.021

126. Atarashi K, Tanoue T, Oshima K, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature*. 2013; 500(7461): 232-236. doi: 10.1038/nature12331

127. Zuo L, Yuan K-T, Yu L, Meng Q-H, Chung PC-K, Yang D-H. Bifidobacterium infantis attenuates colitis by regulating T cell subset responses. *World journal of gastroenterology: WJG*. 2014; 20(48): 18316. doi: 10.3748/wjg.v20.i48.18316

128. Calcinaro F, Dionisi S, Marinaro M, et al. Oral probiotic administration induces interleukin-10 production and prevents spontaneous autoimmune diabetes in the non-obese diabetic mouse. *Diabetologia*. 2005; 48(8): 1565-1575. doi: 10.1007/s00125-005-1831-2

129. Robert S, Gysemans C, Takiishi T, et al. Oral delivery of Glutamic Acid Decarboxylase (GAD)-65 and IL10 by Lactococcus lactis reverses diabetes in recent-onset NOD mice. *Diabetes*. 2014; 63(8): 2876-287. doi: 10.2337/db13-1236

130. Smith PM, Howitt MR, Panikov N, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*. 2013; 341(6145): 569-573. doi: 10.1126/science.1241165

131. Furusawa Y, Obata Y, Fukuda S, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*. 2013; 504(7480): 446-450. doi: 10.1038/nature12721

132. Ferrante RJ, Kubilus JK, Lee J, et al. Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington's disease mice. *The Journal of neuroscience*. 2003; 23(28): 9418-9427.

133. Tao R, de Zoeten EF, Özkaynak E, et al. Deacetylase inhibition promotes the generation and function of regulatory T cells. *Nature medicine*. 2007; 13(11): 1299-1307. doi: 10.1038/nm1652

134. De Zoeten EF, Wang L, Sai H, Dillmann WH, Hancock WW. Inhibition of HDAC9 increases T regulatory cell function and prevents colitis in mice. *Gastroenterology*. 2010; 138(2): 583-594. doi: 10.1053/j.gastro.2009.10.037

135. Manicassamy S, Reizis B, Ravindran R, et al. Activation of β-catenin in dendritic cells regulates immunity versus tolerance in the intestine. *Science*. 2010; 329(5993): 849-853. doi: 10.1126/science.1188510

136. Ganapathy V, Thangaraju M, Prasad PD, Martin PM, Singh N. Transporters and receptors for short-chain fatty acids as the molecular link between colonic bacteria and the host. *Current opinion in pharmacology*. 2013; 13(6): 869-874. doi: 10.1016/j.coph.2013.08.006

137. Blad CC, Tang C, Offermanns S. G protein-coupled receptors for energy metabolites as new therapeutic targets. *Nature Reviews Drug Discovery*. 2012; 11(8): 603-619. doi: 10.1038/nrd3777

138. Singh N, Gurav A, Sivaprakasam S, et al. Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis. *Immunity*. 2014; 40(1): 128-139. doi: 10.1016/j.immuni.2013.12.007

139. Kang SW, Kim SH, Lee N, et al. 1, 25-Dihyroxyvitamin D3 promotes FOXP3 expression via binding to vitamin D response elements in its conserved noncoding sequence region. *The Journal of Immunology*. 2012; 188(11): 5276-5282. doi: 10.4049/jimmunol.1101211

ISSN 2379-6391 Open Journal http://dx.doi.org/10.17140/DROJ-1-109

140. Takiishi T, Ding L, Baeke F, et al. Dietary supplementation with high doses of regular vitamin D3 safely reduces diabetes incidence in nod mice when given early and long-term. *Diabetes*. 2014; DB-131559. doi: 10.2337/db13-1559

141. Kunisawa J, Hashimoto E, Ishikawa I, Kiyono H. A pivotal role of vitamin B9 in the maintenance of regulatory T cells *in vitro* and *in vivo*. *PloS one*. 2012; 7(2): e32094. doi: 10.1371/journal.pone.0032094

142. Kim SV, Xiang WV, Kwak C, et al. GPR15-mediated homing controls immune homeostasis in the large intestine mucosa. *Science*. 2013; 340(6139): 1456-1459. doi: 10.1126/science.1237013

DIABETES RESEARCH

Openventio PUBLISHERS

ISSN 2379-6391 Open Journal http://dx.doi.org/10.17140/DROJ-1-110

Case Report

*Corresponding author
Naoko Aoyagi, DDS
Department of Oral and Maxillofacial Surgery
Hakujyuuji Hospital
3-2-1 Ishimaru, Nishi-ku
Fukuoka 819-0025, Japan
Tel. +81-92-891-2511
Fax: +81-92-881-4491
E-mail: naokoaoyagi@fukuoka-u.ac.jp

Volume 1 : Issue 3
Article Ref. #: 1000DROJ1110

Article History
Received: May 21st, 2015
Accepted: June 24th, 2015
Published: June 25th, 2015

Citation
Aoyagi N, Umemoto G, Nomiyama T, et al. Rapid improvement of blood glucose level after prosthetic mandibular advancement in a patient with diabetes mellitus and obstructive sleep apnea. *Diabetes Res Open J.* 2015; 1(3): 67-71. doi: 10.17140/DROJ-1-110

Rapid Improvement of Blood Glucose Level after Prosthetic Mandibular Advancement in a Patient with Diabetes Mellitus and Obstructive Sleep Apnea

Naoko Aoyagi[1,2*], George Umemoto[2], Takashi Nomiyama[3], Chikara Yoshimura[4], Chikayo Ohta[3], Shiori Miyazaki[3], Kunitaka Murase[3], Ryoko Nagaishi[3], Kentaro Watanabe[5], Toshihiko Yanase[3] and Toshihiro Kikuta[2]

[1]Department of Oral and Maxillofacial Surgery, Hakujyuuji Hospital, Japan

[2]Department of Oral and Maxillofacial Surgery, Faculty of Medicine, Fukuoka University, Fukuoka, Japan

[3]Department of Endocrinology and Diabetes Mellitus, Faculty of Medicine, Fukuoka University, Fukuoka, Japan

[4]Sleep Apnea Center, Kyushu University Hospital, Fukuoka, Japan

[5]Department of Respiratory, Faculty of Medicine, Fukuoka University, Fukuoka, Japan

ABSTRACT

Introduction: Obstructive Sleep Apnea Syndrome (OSAS) is often associated with impaired glucose metabolism. However, the effects of Prosthetic Mandibular Advancement (PMA) on blood glucose levels and insulin resistance remain unclear. Therefore, we assessed the immediate effect of PMA on glycemic control measured using a Continuous Glucose Monitoring System (CGMS) in a patient with Type 2 Diabetes Mellitus (T2DM) and OSAS.
Case presentation: A 77-year-old Japanese woman with T2DM was diagnosed with OSAS with a Respiratory Disturbance Index (RDI) of 39.3. Because the patient did not accept Continuous Positive Airway Pressure (CPAP) therapy, she wore a PMA that advanced the mandible 7 mm forward. Overnight sleep apnea monitoring and CGM were performed before and after wearing the PMA. PMA induced a marked reduction in RDI from 39.3 to 12.8, an increase in the minimum hemoglobin saturation from 78.0% to 87.0%, and a decrease in the number of episodes of oxygen desaturation of >4% below baseline in during the bedtime from 31.3 /h to 12.1 /h. The mean glucose level markedly improved with PMA from 126.1 to 100.5 mg/dL.
Conclusion: The patient with showed improvement in RDI and glucose levels after wearing the PMA overnight. To our knowledge, this is the first case of a patient with OSAS and T2DM showing a beneficial effect of PMA on rapid glycemic control. CGMS may greatly help to promote compliance with the treatment of OSAS in patients with T2DM.

KEYWORDS: Obstructive sleep apnea; Continuous glucose monitoring system; Prosthetic mandibular advancement; Type 2 diabetes mellitus; Insulin resistance.

INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is often associated with the metabolic syndrome[1,2] and also with hypertension, hyperlipidemia, ischemic heart disease, cerebrovascular disease, and impaired glucose metabolism.[3-5] Because of the relationship between OSAS and type 2 diabetes mellitus (T2DM), the effectiveness of continuous positive airway pressure (CPAP) therapy in patients with T2DM has been assessed in many trials, with Hemoblobin A1c (HbA1c) levels improving in some patients.[6,7] However, the effects of prosthetic mandibular advancement (PMA) on blood glucose levels and insulin resistance remain unclear.

DIABETES RESEARCH

Openventio PUBLISHERS

ISSN 2379-6391 Open Journal http://dx.doi.org/10.17140/DROJ-1-110

Continuous glucose monitoring system (CGMS) is a recently developed electronic system designed to continuously monitor subcutaneous glucose concentration in the interstitial fluid. CGMS is a powerful tool for T2DM control because it provides a detailed daily blood glucose profile.[8]

Here we assessed the immediate effect of PMA on glycemic control measured using CGMS in a T2DM patient with OSAS.

CASE REPORT

77-year-old Japanese woman with T2DM [height, 146.6 cm; weight, 64.4 kg; Body Mass Index (BMI), 30.0; Table 1] was admitted to Fukuoka University Hospital, Japan for attending a diabetes mellitus education program. Her Fasting Blood Sugar (FBS) and serum C-peptide levels were 152 mg/dL and 2.22 ng/mL, respectively, indicating a relatively maintained insulin secretory ability. The patient was started on a diet of 1400 kcal/day during her hospital stay. For 14 days, her FBS levels were well controlled; hence insulin therapy was discontinued and only the 1400 kcal/day diet was maintained.

Factors	
Age (year)	77
Gender	F
Height (cm)	146.6
Weight(kg)	64.4
Body mass index (kg/m^2)	30.0
Blood pressure (mmHg)	125/81
Fasting blood sugar (mg/dl)	152
Hemoglobin A1c (NGSP)%	6.2
Low-density lipoprotein (mg/dl)	58
High-density lipoprotein (mg/dl)	38
Triglyceride (mg/dl)	271
Duration of diabetes (year)	2.0
Medicine for diabetes mellitus	Insulin therapy was finished
Other medicines	
Hypertension Antiplatelet Hyperlipemia Anemia	Olmesartan, Medoxomil, Nifedipine, Aspirin, Clopidogrel, Ethyl Icosapentate, Pitavastatin Calcium, ferrous Citrate, Folic Acid

Table 1: Clinical features of the patient with T2DM.

Subsequently, she achieved good glycemic control and did not require other medication, such as oral hypoglycemic agents. On the third hospital day, she was hypoxemic with peripheral oxygen saturation (SpO_2) of 92% measured at rest while breathing room air; however, she did not have subjective symptoms of breathing difficulty. Spirometry testing demonstrated a Forced Vital Capacity (FVC) of 1880 mL (93.5%) and forced expiratory volume in 1 s to FVC ration of 69.2%. An obesity-associated decrease in the movement of the diaphragm possibly caused the SpO_2 drop in the supine position. Because she was suspected to have OSAS, we investigated the degree of sleep apnea using a 2-channel (airflow and SpO_2) portable sleep apnea monitor (LS-120/120S, Fukuda Denshi Co, Ltd, Tokyo, Japan). The SpO_2 decreased and she was finally diagnosed with OSAS with a Respiratory Disturbance Index (RDI) of 39.3. Because she refused CPAP therapy, she was referred to the Department of Oral and Maxillofacial Surgery for PMA. She wore a customized PMA that advanced the mandible 7 mm forward for the evaluation of sleep apnea. During the evaluation, BS levels were monitored using CGMS (iPro2, Medtronic-MiniMed, Northridge, CA, USA), which records the interstitial glucose level every 5 min for up to 72 hr. The CGMS data were analyzed using the CareLink iPro2 software application (Medtronic) and corrected using self-monitoring of blood glucose four times daily. Interstitial glucose levels were monitored for 2 consecutive night: first night without PMA (baseline) and the second night with PMA (12 h of recording each night from 10 pm to 10 am). We obtained 152 measurements on the first night and 143 on the second night. CGMS data were missing for 45 min during the second night (asterisk in Figure1) because of poor contact with the glucose sensor.

PMA resulted in a marked reduction in the RDI from 39.3 to 12.8 PMA (Table 2). With PMA, we observed an increase in the minimum hemoglobin saturation from 78.0% to 87.0%. The mean glucose level (10 pm to 10 am) remarkably improved with PMA from 126.1±24.6 to 100.5±29.6 mg/dL (20.3% decrease, $p<0.001$; Figure 1). Furthermore, the number of episodes of oxygen desaturation of >4% below baseline during the night (10 pm to 6:30 am) decreased from 226/h to 86/h.

Portable Sleep apnea monitor	Without PMA (Baseline)	With PMA
Total scoring time (min)	433	425
Respiratory disturbance index (/h)	39.3	12.8
Apnea/Hypopnea Episode	196/96	57/34
Max Period of Apnea Lasting (sec)	71	47
4% Oxgen desaturation index (times)	226	86
minSpO_2 (%)	78	87
meanSpO_2 (%)	94	94
CGMS		
mean blood glucose (mg/dl) ±SD (min-max)	126.1±24.6 (108-200)	100.5±29.6 (60-153)

Table 2: Results of the portable sleep apnea monitor and CGMS.

DISCUSSION

OSAS is characterized by repetitive episodes of upper airway obstruction occurring during sleep, generally associated with a decrease in blood oxygen saturation.[9] Furthermore, OSAS is associated with insulin resistance and T2DM.[10,11] Tamura, et al.[11] reported that Impaired Glucose Tolerance (IGT) was observed in 60.5% patients with sleep apnea (30.2% with T2DM as well). Another study reported that T2DM was pres-

ISSN 2379-6391 Open Journal http://dx.doi.org/10.17140/DROJ-1-110

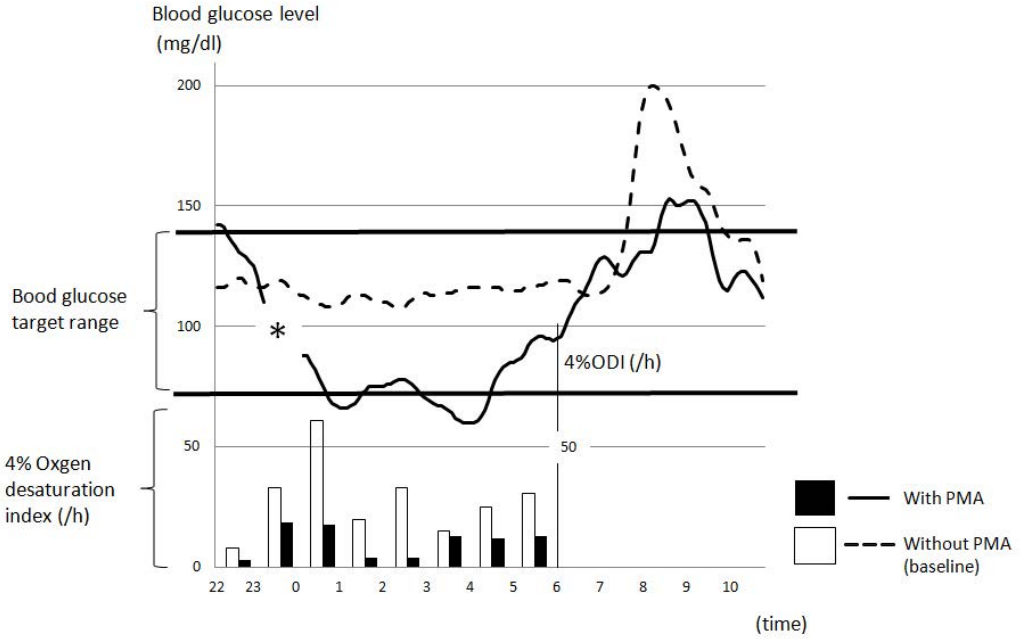


Figure 1: Line and bar graphs showing changes in the mean glucose level using the CGMS during the night (10 pm to 10 am) and the number of episodes of oxygen desaturation of >4% below baseline /h during the night (10 pm to 6:60 am) respectively. *Glucose levels were not monitored from 11:15 pm to midnight because of difficulty with a sensor.

ent in 30.1% patients with OSAS and 13.9 snorers; IGT was diagnosed in 20.0% patients with OSAS and 13.9% non-apneic snorers.[12] In addition, the study reported that insulin sensitivity decreased when the severity of sleep apnea increased. Different studies have indicated that BMI is the major factor for insulin resistance in patients with OSAS.[13,14] However, even after controlling for obesity and other confounding factors of insulin resistance, the apnea-hypopnea index and/or a minimum SpO_2 were reported to be still associated with fasting insulin level and Homeostasis model assessment of insulin resistance (HOMA-IR).[13] Diabetic control is generally because of increase in insulin secretion, insulin sensitivity, or both. In our case, although the serum C-peptide level was normal, HOMA-IR was not performed because of the insulin therapy in the initial stage, thus impeding the estimation of the presence or absence of insulin resistance. Hence, the reason for the rapid FBS improvement during the night with PMA remains unclear.

Spiegel K, et al.[15] assessed carbohydrate metabolism in 11 young men who had their sleep duration restricted to 4 h/night for 6 nights. Glucose tolerance was lower in participants deprived of sleep than in those completely rested; in addition, evening cortisol levels were significantly elevated in the sleep-deprived participants.[15] Another study found a decrease in oxyhemoglobin saturation, which was induced when the patients were awake, and suggested that this intermittent hypoxia was associated with a decrease in insulin sensitivity.[16] In a recent study, after 2 nights of sleep fragmentation, decreased insulin sensitivity and glucose effectiveness was observed, i.e, the ability of glucose to mobilize itself independently in response to insulin had decreased.[17] The duration of T2DM in our patient was only 2 years, and she had no subjective symptoms of OSAS, including daytime sleepiness or snoring. Obesity-induced desaturation and OSAS might be partly associated with the occurrence of T2DM in this patient.

A previous study evaluated the insulin resistance of 40 patients with OSAS before and after CPAP therapy based on hyperinsulinemic-euglycemic clamp studies, and found that insulin resistance improved after CPAP therapy, particularly in non-obese patients.[18] Using a 72-h CGMS, Babu, et al.[6] studied the changes in interstitial glucose levels and measured HbA1c levels in 25 patients with T2DM before and after CPAP therapy for OSAS. After CPAP therapy, they observed a significant decrease in both 1-h postprandial glucose and HbA1c levels after CPAP therapy.[6] Furthermore, a significant correlation between decrease in HbA1c levels and the duration of CPAP therapy was observed in patients who used CPAP for more than 4 h/day. Our case showed a decrease in FBS levels similar to that found in studies using CPAP therapy.[6,18] This result suggests that PMA may have an equal effect on blood glucose levels as CPAP therapy.

PMA prevents upper airway collapse in patients with OSAS. Recent American Academy of Sleep Medicine guidelines concluded that oral appliances are less effective than CPAP but are a reasonable alternative for patients with mild to moderate OSAS in specific situations.[19,20] To the best of our knowledge, the present case report is the first showing the impact of PMA on glycemic control assessed using CGMS.

Improvement of intermittent hypoxia by wearing PMA

during the nights may have had a beneficial effect on glycemic control in our case. Any significant improvement of RDI and oxygen saturation levels achieved with PMA may have an immediate effect on blood glucose levels in a patient with T2DM. Because here we have reported only one such case, further investigations are required to confirm whether the beneficial effect of PMA can be observed in a large number of T2DM patients complicated with OSAS.

In conclusion, our T2DM patient with OSAS showed improvement in RDI and glucose levels after wearing a PMA during the night. This case suggests an immediate effect of the PMA on glycemic control. The results obtained using CGMS in this case, regarding the effect of PMA on glycemic control support the importance of adequate treatment in T2DM patients with OSAS. Further discussion of such benefits can help providers promote compliance in patients with T2DM.

CONFLICTS OF INTEREST: None.

CONSENT

The patient has provided written permission for publication of the case details.

REFERENCES

1. Chin K, Oga T, Takahashi K, et al. Associations between obstructive sleep apnea, metabolic syndrome, working population in Japan. *Sleep.* 2010; (33): 89-95.

2. Coughlin SR, Mawdlsey L, Mugarza JA, et al. Obstructive sleep apnea is independently associated with an increased prevalence of metabolic syndrome. *Eur Heart J*. 2004; 25: 735-741. doi: 10.1016/j.ehj.2004.02.021

3. Tiihonen M, Partinen M, Narvanen S. The severity of obstructive sleep apnea is associated with insulin resistance. *J Sleep Res*. 1993; 2: 56-61.

4. Vgontzas AN, Papanicolaou DA, Bixler EO, et al. Sleep apnea and daytime sleepiness and fatigue: relation to visceral obesity, insulin resistance, and hypercytokinemia. *J Clin Endocrinol Metab*. 2000; 85: 1151-1158.

5. Punjabi MN, Sorkin JD, Katzel LI, et al. Sleep-disordered breathing and insulin resistance in middle-aged and overweight men. *Am J Respir Crit Care Med.* 2002; 165: 677-678. doi: 10.1164/ajrccm.165.5.2104087

6. Babu A, Herdegen J, Fogelfeld L, et al. Type 2 diabetes, glycemic control, and continuous positive airway pressure in obstructive sleep apnea. *Arch Intern Med*. 2005; 165: 447-452. doi: 10.1001/archinte.165.4.447

7. Hassaballa H, Tulaimat A, Herdegen J, et al. The effect of continuous positive airway pressure on glucose control in diabetic patients with severe obstructive sleep apnea. *Sleep Breath*. 2005; 9: 176-180. doi: 10.1007/s11325-005-0033-y

8. Mastrototaro J. The MiniMed Continuous glucose monitoring system (CGMS). *J Pediatr Endocrinol Metab*. 1999; 12: 751-758.

9. ASDA-Diagnostic Classification Steering Committee. The International Classification of Sleep Disorders. *Diagnostic and Coding Manual,* 2nd ed. Lawrence, KS: Allen Press Inc, Washington DC, 1997.

10. Peled N, Kassirer M, Shitrit D, et al. The association of OSAS with insulin resistance, inflammation and metabolic syndrome. *Respir Med*. 2007; 101: 2007.

11. Tamura A, Kawano Y, Watanabe T, et al. Relationship between the severity of obstructive sleep apnea and impaired glucose metabolism in patients with obstructive sleep apnea. *Respir Med*. 2008; 102: 1412-1416. doi: 10.1016/j.rmed.2008.04.020

12. Meslier N, Gagnadoux F, Giraud P, et al. Impaired glucose-insulin metabolism in males with obstructive sleep apnoea syndrome. *Eur Respir J*. 2003; 22: 156-160. doi: 10.1183/09031936.03.00089902

13. Ip MS, Lam B, Ng MM, et al. Obstructive sleep apnea is independently associated with insulin resistance. *Am J Respir Crit Care Med*. 2002; 165: 670-676. doi: 10.1164/ajrccm.165.5.2103001

14 Otake K, Sasanabe R, Hasegawa R, et al. Glucose intolerance in Japanese patients with obstructive sleep apnea. *Intern Med*. 2009; 48: 1863-1868. doi: 10.2169/internalmedicine.48.2465

15. Spiegel K, Leproult R, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. *Lancet*. 1999; 354: 1435-1439. doi: 10.1016/S0140-6736(99)01376-8

16. Louis M, Punjabi NM. Effects of acute intermittent hypoxia on glucose metabolism in awake healthy volunteers. *J Appl Physiol*. 2009; 106: 1538-1544. doi: 10.1152/japplphysiol.91523.2008

17. Stamatakis KA, Punjabi NM. Effects of sleep fragmentation on glucose metabolism in normal subjects. *Chest*. 2010; 137: 95-101. doi: 10.1378/chest.09-0791

18. Harsch IA, Schahin SP, Radespiel-Troger M, et al. Continuous positive airway pressure treatment rapidly improves insulin sensitivity in patients with obstructive sleep apnea syndrome. *Am J Respir Crit Care Med*. 2004; 15: 156-162. doi: 10.1164/rccm.200302-206OC

19. Ferguson KA, Cartwright R, Rogers R, et al. Oral appliances

ISSN 2379-6391 Open Journal http://dx.doi.org/10.17140/DROJ-1-110

for snoring and obstructive sleep apnea: a review. *Sleep*. 2006; 292: 244-262.

20. Kushida CA, Morgenthaler TI, Littner MR, et al. American Academy of Sleep Practice parameters for the treatment of snoring and obstructive sleep apnea with oral appliances. an update for 2005. *Sleep*. 2006; 292: 240-243.

DIABETES RESEARCH

Openventio PUBLISHERS

ISSN 2379-6391 Open Journal http://dx.doi.org/10.17140/DROJ-1-111

Opinion

Corresponding author
Yuji Aoki, MD, PhD
Department of Internal Medicine
National Hospital Organization
Matsumoto Medical Center
Matsumoto Hospital
2-20-30 Minami, Murai
Matsumoto 399-8701, Japan
Tel. 0263 58 4567
Fax: 0263 86 3183
E-mail: yaoki55@nifty.com

Volume 1 : Issue 3
Article Ref. #: 1000DROJ1111

Article History
Received: June 25th, 2015
Accepted: July 1st, 2015
Published: July 1st, 2015

Citation
Aoki Y, Administration of sodium-glucose co-transporter2 inhibitors could accelerate dehydration in poorly-controlled diabetic patients, proposing an option not to increase glucosuria but to decrease carbohydrate intake during hyperglycemia. *Diabetes Res Open J*. 2015; 1(3): 72-74. doi: 10.17140/DROJ-1-111

Administration of Sodium-Glucose Co-transporter 2 Inhibitors Could Accelerate Dehydration in Poorly-Controlled Diabetic Patients, Proposing an Option not to Increase Glucosuria but to Decrease Carbohydrate Intake during Hyperglycemia

Yuji Aoki*

Department of Internal Medicine, National Hospital Organization Matsumoto Medical Center Matsumoto Hospital, Japan

ABSTRACT

Sodium-glucose co-transporter 2 (SGLT2) inhibitors, a new class of anti-diabetic agents, have been recently approved for treatment of type 2 diabetes. It was unexpected that possible adverse effects of SGLT2 inhibitors, including fatal events, were reported frequently soon after the first one was marketed in April 2014 in Japan. In poorly-controlled diabetic patients, pre-existing osmotic diuresis is supposed to be augmented by the administration of SGLT2 inhibitors, possibly leading to an acceleration of their dehydration in spite of amelioration of hyperglycemia. It may be reasonable that not only water but a small amount of salt needs to be supplemented with, if necessary, to prevent plasma volume depletion with salt loss. Otherwise, it seems to be a plausible option for such patients to decrease carbohydrate intake by 50 to 100 g of carbohydrate per day during hyperglycemia, instead of excreting a similar amount of glucose into urine with SGLT2 inhibitors.

KEYWORDS: SGLT2 inhibitors; Glucosuria; Osmotic diuresis; Dehydration; Carbohydrate intake.

Sodium-glucose co-transporter 2 (SGLT2) inhibitors, a new class of anti-diabetic agents, have been recently approved for treatment of type 2 diabetes, since dapagliflozin was first approved by the European Medicines Agency in November 2012. SGLT2 inhibitors decrease hyperglycemia independently of insulin by increasing urinary glucose excretion through the inhibition of glucose reabsorption in the proximal renal tubule. They have some advantages including modest weight loss, low risk of hypoglycemia and mild decrease of blood pressure.[1] However, it is reported that Canagliflozin cardiovascular assessment study (CANVAS) showed an increase of cardiovascular events during the first 30 days in patients who received canagliflozin,[2] the first SGLT2 inhibitor approved in the United States in March 2013. In Japan, six SGLT2 inhibitors have been approved since January 2014. It is a matter of concern that the Japan Diabetes Society announced an alert regarding proper use of SGLT2 inhibitors, twice in June and August 2014. It was unexpected that possible adverse effects of SGLT2 inhibitors were reported frequently soon after the first one was marketed in April 2014. Finally, dehydration, which seemed to be occasionally linked to fatal adverse events, was added as a severe adverse effect to a package insert in January 2015.

Familial renal glucosuria with normoglycemia is often referred to as a natural model for SGLT2 inhibition. Plasma volume depletion resulting from osmotic diuresis was indicated by activation of the renin-angiotensin-aldosterone system in cases of severe renal glucosuria (>10 g/1.73m^2/24h) with a favourable prognosis.[3,4] Such activation has been shown after the administration of empagliflozin, another SGLT2 inhibitor, in type 1 diabetic patients.[5] In line

with plasma volume depletion, salt loss is suggested to occur early after the administration of SGLT2 inhibitors,[6,7] reaching to a new balance of total body salt. The diuretic effect of dapagliflozin causes small but significant increases in urine volume, blood urea nitrogen and hematocrit without an increase in serum sodium.[8] As Haas, et al.[9] described, no hypernatremia as a trigger for thirst may explain why, in particular, elderly patients do not develop sufficient thirst to compensate for water loss and consequently tend to have dehydration, unstable pressure or syncope.

In poorly-controlled diabetic patients, pre-existing osmotic diuresis is supposed to be augmented by the administration of SGLT2 inhibitors, possibly leading to an acceleration of their dehydration in spite of amelioration of hyperglycemia. In the case of parenteral administration of mannitol, an osmotic diuretic, initial volume expansion and subsequent hypovolemia is known as one of its adverse effects.[10] From this point of view, it is inferred that hypovolemia is more likely to occur due to osmotic diuresis without an increase in blood glucose (and possibly sodium[8,11]) level to retain water following the administration of SGLT2 inhibitors, even if drinking water is recommended. If this is true, use of SGLT2 inhibitors should be avoided in poorly-controlled diabetic patients. The amelioration of hyperglycemia by treatment with SGLT2 inhibitors might mislead into overestimating the safety of their use. Since familial renal glucosuria with normoglycemia usually has no apparent clinical problems,[3,4] it may need to be determined from which level of HbA1c it becomes safer to use SGLT2 inhibitors for diabetic patients of different ages with the variety of vascular complications.

As in the statement of "Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach",[12] SGLT2 inhibitors with a diuretic effect should be used cautiously in the elderly, in any patient already on a diuretic, and in anyone with a tenuous intravascular volume status. It may be reasonable that not only water but a small amount of salt needs to be supplemented with, if necessary, to prevent plasma volume depletion with salt loss. Otherwise, it seems to be a plausible option for such patients to decrease carbohydrate intake by 50 to 100 g of carbohydrate per day during hyperglycemia, instead of excreting a similar amount of glucose into urine with SGLT2 inhibitors. Such carbohydrate restriction seems to be readily accepted for diabetic patients educated on carbohydrate counting.[13,14] Despite the inconclusive results of the studies evaluating the effect of differing percentages of carbohydrates, evidence exists that total amount of carbohydrate eaten is the primary predictor of glycemic response.[15] Feinman, et al.[16] claim that evidence supports the use of low-carbohydrate diets as the first approach to treating type 2 diabetes and as the most effective adjunct to pharmacology in type 1 diabetes. Then, conversely, adjunctive use of SGLT2 inhibitors may be also effective in type 1 diabetes.[17] Further studies should be needed towards a better understanding of the benefit to risk ratio of treatment with SGLT2 inhibitors, a unique and anticipated anti-diabetic agent.

CONFLICTS OF INTEREST

The author declares that he has no conflicts of interest.

REFERENCES

1. Mikhail N. Place of sodium-glucose co-transporter type 2 inhibitors for treatment of type 2 diabetes. *World J Diabetes*. 2014; 5: 854-859. doi: 10.4239/wjd.v5.i6.854

2. Vasilakou D, Karagiannis T, Athanasiadou E, et al. Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes: a systematic review and meta-analysis. *Ann Intern Med*. 2013; 159: 262-274. doi: 10.7326/0003-4819-159-4-201308200-00007

3. Scholl-Buergi S, Santer R, Ehrich JHH. Long-term outcome of renal glucosuriatype 0: the original patient and his natural history. *Nephrol Dial Transplant*. 2004; 19: 2394-2396. doi: 10.1093/ndt/gfh366

4. Calado J, Sznajer Y, Metzger D, et al. Twenty-one additional cases of familial renal glucosuria: absence of genetic heterogeneity, high prevalence of private mutations and further evidence of volume depletion. *Nephrol Dial Transplant*. 2008; 23: 3874-3879. doi: 10.1093/ndt/gfn386

5. Cherney DZI, Perkins BA, Soleymanlou N, et al. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. *Circulation*. 2014; 129: 587-597. doi: 10.1161/CIRCULATIONAHA.113.005081

6. Katsuno K, Fujimori Y, Takemura Y, et al. Sergliflozin, a novel selective inhibitor of low-affinity sodium glucose reabsorption and modulates plasma glucose level. *J Pharmacol Exp Ther*. 2007; 320: 323-330. doi: 10.1124/jpet.106.110296

7. Lin B, Koibuchi N, Hasegawa Y, et al. Glycemic control with empagliflozin, a novel selective SGLT2 inhibitor, ameliorates cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice. *Cardiovasc Diabetol*. 2014; 13: 148. doi: 10.1186/s12933-014-0148-1

8. List JF, Woo V, Morales E, Tang W, Fiedorek FT. Sodium-glucose cotransport inhibition with dapagliflozin in type 2 diabetes. *Diabetes Care*. 2009; 32: 650-657. doi: 10.2337/dc08-1863

9. Haas B, Eckstein N, Pfeifer V, Mayer P, Hass MDS. Efficacy, safety and regulatory status of SGLT2 inhibitors: focus on canagliflozin. *Nutr Diabetes*. 2014; 4: e143. doi: 10.1038/nutd.2014.40

10. Shawkat H, Westwood MM, Mortimer A. Mannitol: a review of its clinical uses. *Contin Educ Anaesth Crit Care Pain*. 2012; 12(2): 82-85. doi: 10.1093/bjaceaccp/mkr063

11. Bernstein LM, Blumberg B, Arkin MC. Osmotic diuretic treatment of refractory edema. *Circulation*. 1958; 17; 1013-1020. doi: 10.1161/01.CIR.17.6.1013

12. Inzucchi SE, Bergenstal RM, Buse JB, et al. Management of hyperglycaemia in type 2 diabetes, 2015: a patient-centered approach. Update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetologia.* 2015; 58: 429-442.

13. Warshaw HS, Bolderman KM. Practical carbohydrate counting. 2nd ed. Alexandria, VA: American Diabetes Association. 2008.

14. Aoki Y, Onzuka M. Glycemic variations after ingestion of different carbohydrate-containing foods assessed by continuous glucose monitoring in healthy and diabetic individuals in daily life. *Diabetes Res Open J.* 2015; 1(2): 41-47.

15. Evert AB, Boucher JL, Cypress M, et al. Nutrition therapy recommendations for the management of adults with diabetes. *Diabetes Care*. 2013; 36: 3821-3842. doi: 10.2337/dc13-2042

16. Feinman RD, Pogozelski WK, Astrup A, A et al. Dietary carbohydrate restriction as the first approach in diabetes management: critical review and evidence base. *Nutrition*. 2015; 31: 1-13. doi: 10.1016/j.nut.2014.06.011

17. Perkins BA, Cherney DZI, Partridge H, et al. Sodium-glucose cotransporter 2 inhibition and glycemic control in type 1 diabetes: results of an 8-week open-label proof-of-concept trial. *Diabetes Care*. 2014; 37: 1480-1483. doi: 10.2337/dc13-2338

DIABETES RESEARCH

Openventio PUBLISHERS

ISSN 2379-6391 Open Journal http://dx.doi.org/10.17140/DROJ-1-112

Opinion

*Corresponding author
Dimiter Dimitrov, MD
Consultant Endocrinologist
Diavita Ltd
Varna, Bulgaria
E-mail: dimiter.v.dimitrov@gmail.com

Volume 1 : Issue 3
Article Ref. #: 1000DROJ1112

Article History
Received: June 25th, 2015
Accepted: July 1st, 2015
Published: July 1st, 2015

Citation
Dimitrov D. Do we need new therapies for diabetes?. *Diabetes Res Open J*. 2015; 1(3): 75-76. doi: 10.17140/DROJ-1-112

Do We Need New Therapies For Diabetes?

Dimiter Dimitrov*

Consultant Endocrinologist, Diavita Ltd, Varna, Bulgaria

Diabetes research and practice cluster (drug developers, payers, regulators and physicians) often (and especially in recent times) question the need of new therapies. Why would we need new therapies nowadays, when we have 9 classes (insulins, sulfonylureas, biguanides, meglitdes, thiazolidnediones, alpha-glucosidase inhibitors, DPP-4 inhibitors, SGLT2 inhibitors and GLP-1 receptor agonists),[1] rapidly increasing number of biosimilars[2] and uncountable number of generics? Reasonable question. Does it make sense to invest billions of dollars in messy global cardiovascular outcomes trials (as requested by diabetes drug development guidelines[3]) or to play love attraction games with payers[4] so they "fall in love" with the "new" pill (most often combo with the good old metformin)?

Well….I believe someone is missing in this picture. Obviously, those four players forget the Patient. And the ability to ask a bit more simple and rational questions, such as "*Do we have adequate glycemic control of diabetes in the presence of all those therapies*"?

I know that such question would add "noise" in the discussion from the different parties returning back the ball to the patients, who do not have proper life style and as well as knowledge on the condition.[5,6] Question is: Could they?

To avoid entering a philosophical thoughtfulness that we do not live a perfect world, I will answer directly the main question of this Opinion. Yes, we desperately need breakthrough therapies for diabetes. Not therapies that mimic the current ones (long or ultra fast acting versions, combos or biosimilars). We need therapies that do not complicate the natural way of thinking (and living) by adding the next complex scheme (currently named "personalized").

At the end of the day, diabetes per se is loss of pancreas function and the only way to restore this loss is to develop therapies that restore pancreas cells.

Two companies pioneer the field: ViaCyte and Mesoblast. Though their approach is different they both target regenerative stem cells and I believe this is the future. Couple of other companies use iPS cells for different indications (just to mention BioTime, Inc [oncology and orthopaedics], Vericel Corporation [rheumatology and cardiomyopathy] among many others).

Major concern for all anti-diabetic therapies is cardiovascular safety profile. As a matter of fact, previous treatment recommendations for "intensified" control of diabetes pushed medical practitioners to lower blood glucose beyond physiological equilibrium[8] and this led to an end of decade era of dogmatic schemes – however a positive outcome – leading the current guidelines to more flexible framework.

ViaCyte has entered Phase II programme for T1DM using implantable subcutaneous devise,[9] following positive Proof of Concept studies.

While Mesoblast Allogeneic Mesenchymal Precursor Cells (MPCs) not only excel positive on glycaemia; they also promote additional heart and renal protective effects, which will be further tested in global Phase III trials.[10]

CONFLICTS OF INTEREST

The author declares no conflict of interest.

REFERENCES

1. American Diabetes Association. Standards of medical care in diabetes-2015 abridged for primary care providers. *Clin Diabetes.* 2015; 33(2): 97-111. doi: 10.2337/diaclin.33.2.97

2. Biosimilar insulins. Biosimilars-what you need to know? Diabetes UK. Available at: https://www.diabetes.org.uk/About_us/News/Biosimilars-update/ 2014; Accessed 2015.

3. Shuren J. Guidance for industry diabetes mellitus-evaluating cardiovascular risk in new antidiabetic therapies to treat type 2 diabetes. Available at: https://www.federalregister.gov/articles/2008/12/19/E8-30086/guidance-for-industry-on-diabetes-mellitus-evaluating-cardiovascular-risk-in-new-antidiabetic 2008; Accessed 2015.

4. Mozeson M, Das N. The Pharma/Payer Relationship. *The Pulse: The Wharton Health Care Journal.* 2010.

5. Shuman J. Strategies for improved treatment adherence in type 2 diabetes. Available at: http://www.medpagetoday.com/resource-center/diabetes/Strategies-Improved-Treatment-Adherence-Type-2-Diabetes/a/31638 2012; Accessed 2015.

6. Stuart BC, Dai M, Xu J, E Loh FH, S Dougherty J. Does good medication adherence really save payers money? *J Med Care.* 2015; 53(6): 517-523. doi: 10.1097/MLR.0000000000000360

7. Schmieder RE, Gitt AK, Koch C, et al. Achievement of individualized treatment targets in patients with comorbid type-2 diabetes and hypertension: 6 months results of the DIALOGUE registry. *BMC EndocrDisord*. 2015; 15: 23. doi: 10.1186/s12902-015-0020-7

8. Psaty BM, Furberg CD. Rosiglitazone and cardiovascular risk. *N Engl J Med.* 2007; 356: 2522-2524. doi: 10.1056/NEJMe078099

9. ViaCyte. A service of the US National Institutes of Health. Clinical Trails. Available at: https://clinicaltrials.gov/ct2/show/NCT02239354?term=viacyte&rank=1 2014; Accessed 2015.

10. Positive Diabetic Nephropathy Trial Results presented at ADA 2015 Meeting. Available at: http://mesoblast.com/news-and-media/news-announcements 2015; Accessed 2015.

DIABETES RESEARCH

Openventio PUBLISHERS

ISSN 2379-6391 Open Journal http://dx.doi.org/10.17140/DROJ-1-113

Letter to the Editor

Corresponding author
Hideshi Okada, MD, PhD
Department of Emergency and Disaster Medicine, Gifu University Graduate School of Medicine, 1-1 Yanagido Gifu 501-1194, Japan
Tel. +81-58-230-6448
Fax: +81-58-230-6451
E-mail: hideshi@gifu-u.ac.jp

Volume 1 : Issue 3
Article Ref. #: 1000DROJ1113

Article History
Received: June 30th, 2015
Accepted: July 10th, 2015
Published: July 13th, 2015

Citation
Okada H, Takemura G, Suzuki K, et al. Substitution of chronic insulin therapy with dipeptidyl peptidase-4 inhibitors and sodium-glucose co-transporter-2 inhibitors. *Diabetes Res Open J.* 2015; 1(3): 77-78. doi: 10.17140/DROJ-1-113

Substitution of Chronic Insulin Therapy with Dipeptidyl Peptidase-4 Inhibitors and Sodium-Glucose Co-transporter-2 Inhibitors

Hideshi Okada[1,2*], Genzou Takemura[2,3], Kodai Suzuki[1], Nagisa Miyazaki[2,4], Eiji Kuroda[5], Yoko Ito[4], Toshihiko Chikaishi[4], Hiroaki Ushikoshi[1] and Shinji Ogura[1]

[1]Department of Emergency and Disaster Medicine, Gifu University Graduate School of Medicine, Gifu 501-1194, Japan

[2]Department of Internal Medicine, Chikaishi Clinic, Gifu, Japan

[3]Department of Internal Medicine, Asahi University, Hozumi 1851-1, Japan

[4]Department of Nephrology, Gifu University Graduate School of Medicine, Gifu 501-1194, Japan

[5]Department of Internal Medicine, Kaizu Medical Association Hospital, Kaizu, Japan

Insulin is a very useful and widely used treatment for diabetes. Temporary insulin therapy improves glucose toxicity due to improved β-cell function of the pancreas. Upon achieving glycemic control, insulin treatment could be discontinued and substituted with oral hypoglycemic agents. Nevertheless, insulin therapy is associated with side effects such as hypoglycemia, allergic reactions, and angioneurotic edema. Over this past decade, there have been rapid advances in diabetes treatment, including the introduction of Dipeptidyl peptidase-4 (DPP-4) inhibitors and Sodium-glucose cotransporter-2 (SGLT2) inhibitors. We present here the case of a patient with type 2 diabetes who discontinued insulin therapy after more than 20 years by switching to oral hypoglycemic agents including a DPP-4 inhibitor and a SGLT2 inhibitor.

A 64-year-old man with type 2 diabetes was being treated with Lispro Mix 50 insulin twice daily. He was started on subcutaneous insulin 20 years ago. He also has hypertension and hyperlipidemia, and visits the home clinic once a month. He consulted the clinic because he strongly wanted to discontinue insulin therapy due to his work situation. At the time, he was taking Lispro Mix 50 insulin twice daily (morning: 10U, evening: 6U) and his HbA1c was 7.3%. His body weight was 47.0 kg, height was 160 cm, and body mass index was 18.4 kg/m^2. His blood pressure was 118/68 mm Hg and his pulse rate was 72 beats per minute. After evaluation of his condition, insulin therapy was discontinued and oral therapy consisting of glimepiride 1 mg/day, teneligliptin 20 mg/day, and canagliflozin 100 mg/day was started. Serum C-peptide and HbA1c were 0.9 ng/ml and 7.3% respectively three months later.

In theory, the dose of insulin should be reduced gradually when oral hypoglycemic agents are added and insulin therapy is being discontinued. However, the patient demanded immediate discontinuation of insulin therapy. Since his insulin dose was relatively low, 0.3 U/kg, insulin was discontinued, and oral hypoglycemic agents were started at once after a comprehensive review of the risks and benefits of a sudden change in therapy.

Early introduction of short-term insulin therapy is more protective for the beta cells of pancreas than oral hypoglycemic therapy in patients with type 2 diabetes. Surprisingly, in the present case, although the duration of insulin therapy was over 20 years, serum C-peptide, an indicator of insulin secretion, was 0.9 ng/ml, which is not too low. This may be explained by the following: 1) glimepiride increases insulin release from the β-cells of the pancreas, and

ISSN 2379-6391 Open Journal http://dx.doi.org/10.17140/DROJ-1-113

2) DPP-4 inhibitors increase incretin levels, which suppresses glucagon release, thereby increasing insulin secretion and decreases blood glucose levels.

SGLT2 inhibitors reduce hyperglycemia by increasing urinary glucose excretion independent of insulin secretion or action. In addition, it has been reported that SGLT2 inhibitors increase glucose–dependent insulin secretion by improving β-cell function of the pancreas.[1]

Based on the empiric evidence, DPP4 inhibitors and SGLT2 inhibitors are effective in patients who secrete less insulin after long-term insulin therapy since they do not have a direct effect on insulin secretion.

CONFLICTS OF INTEREST: None.

CONSENT

The patient has provided the informed written consent for this case to be published.

REFERENCE

1. Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose co-transporter 2 inhibition in type 2 diabetic patients. *J Clin Invest.* 2014; 124(2): 499-508. doi: 10.1172/JCI72227