Targeting dysfunctional beta-cell signaling for the potential treatment of type 1 diabetes mellitus

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Impact statement

The expanding investigation of beta-cell therapeutic targets for the treatment and prevention of type 1 diabetes mellitus is fundamentally relevant and timely. This review summarizes the overall scope of research into novel type 1 diabetes mellitus therapeutics, highlighting weaknesses or caveats in current clinical trials as well as describing potential new targets to pursue. More specifically, signaling proteins that act as modulators of beta-cell function, survival, and replication, as well as immune infiltration may need to be targeted to develop the most efficient pharmaceutical interventions for type 1 diabetes mellitus. One such beta-cell signaling pathway, mediated by the alpha subunit of the heterotrimeric G_z protein ($G\alpha_z$), is discussed in more detail. The work described here will be critical in moving the field forward as it emphasizes the central role of the beta-cell in type 1 diabetes mellitus disease pathology.

Abstract

Since its discovery and purification by Frederick Banting in 1921, exogenous insulin has remained almost the sole therapy for type 1 diabetes mellitus. While insulin alleviates the primary dysfunction of the disease, many other aspects of the pathophysiology of type 1 diabetes mellitus are unaffected. Research aimed towards the discovery of novel type 1 diabetes mellitus therapeutics targeting different cell signaling pathways is gaining momentum. The focus of these efforts has been almost entirely on the impact of immunomodulatory drugs, particularly those that have already received FDA-approval for other autoimmune diseases. However, these drugs can often have severe side effects, while also putting already immunocompromised individuals at an increased risk for other infections. Potential therapeutic targets in the insulin-producing beta-cell have been largely ignored by the type 1 diabetes mellitus field, save the glucagon-like peptide 1 receptor. While there is preliminary evidence to support the clinical exploration of glucagon-like peptide 1 receptor-based drugs as type 1 diabetes mellitus adjuvant therapeutics, there is a vast space for other putative therapeutic targets to be explored. The alpha subunit of the heterotrimeric G_z protein ($G\alpha_z$) has been shown to promote beta-cell inflammation, dysfunction, death, and failure to replicate in the context of diabetes in a number of mouse models. Genetic loss of $G\alpha_z$ or inhibition of the $G\alpha_z$ signaling pathway through dietary interventions is protective against the development of insulitis and hyperglycemia. The multifaceted effects of $G\alpha_z$ in regards to beta-cell health in the context of diabetes make it an ideal therapeutic target for further study. It is our belief that a low-risk, effective therapy for type 1 diabetes mellitus will involve a multidimensional approach targeting a number of regulatory systems, not the least of which is the insulin-producing beta-cell.

Keywords: Diabetes, G protein, GPCR, islet, signaling, therapy

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Introduction

In 2013, T1DM in the U.S. was estimated to affect 3 in 1000 civilians, and 2017 National Diabetes Statistics Report from the Center for Disease Control (CDC) suggests over 1.5 million people are currently diagnosed with T1DM.[1,2] In 95 years of prescribing therapeutic treatments for individuals with type 1 diabetes mellitus (T1DM), the medical community has had essentially one option: insulin.[3] At first glance, treatment with exogenous insulin seems to be the best choice for T1DM management, as the hallmark feature of the disease is autoimmune destruction of the insulin-producing beta-cells in the pancreatic Islets of

Langerhans. And there is no question that, until we can replace an individual's beta-cells effectively and durably, insulin therapy will be an absolute requirement for T1DM patients. Yet, despite its seemingly obvious and clear-cut efficacy, the use of insulin to correct pathological hyperglycemia is difficult to achieve and maintain over a lifetime. And poor glucose control over a lifetime significantly increases the risk of comorbidities, such as retinopathy, neuropathy, cardiovascular disease, and many more. As the understanding of the molecular bases behind T1DM pathophysiology grows, the opportunity to expand the scope of T1DM therapeutic drug targets expands accordingly. In accordance with this notion, there is strong interest in developing targeted therapeutics that could improve the mass or function of beta-cells in the T1DM state.

T1DM as an autoimmune disease

T1DM used to be defined simply as an autoimmune disease targeting the destruction of the insulin-producing beta-cells. At some point in T1DM, the remaining functional beta-cell mass, if any, is insufficient to control blood glucose levels. Generally, the onset of T1DM follows maladaptation to "stressors," a variety of which have been identified and are being investigated, including environmental and genetic risk factors. There exist a number of putative environmental factors that influence T1DM onset and pathogenesis, varying in their strength of supporting evidence. These factors range from enteroviral infection to nutritional components, such as cow's milk and gluten.[4] In addition, almost 60 genetic loci have been identified to associate with T1DM risk. The most studied and strongly associated loci encode variants of the major histocompatibility complexes, also referred to as human leukocyte antigen (HLA) in humans. HLAs are responsible for appropriate antigen presentation by all nucleated cells.[5,6] The HLA region variants account for about half of familial genetic risk, while all identified loci combined account for 80%–85% of the inheritable risk for T1DM.[7,8] Regardless of the underlying etiology, though, an inability to adapt results in the autoimmune destruction of pancreatic beta-cells, the sole producers of insulin in the body.[9–16] Although it has not been delineated whether initiation occurs as an inappropriate immune response or inappropriate signaling from the beta-cell, both play fundamental roles in the onset and acceleration of the disease state.

It is well-accepted that innate and adaptive immune cells facilitate disease progression, driven primarily by auto-reactive CD4+ and CD8+ T-cells and facilitated by auto-antigen presentation by B cells.[17] Current research suggests insulin, glutamic acid decarboxylase 65 (GAD65), and islet tyrosine phosphatase 2 (IA-2) are the three major antigens targeted by autoantibodies.[18] Invasion by innate immune cells, primarily macrophages, followed by antigen presentation and activation of autoreactive T-cells leads to immune infiltration of the pancreatic islet, further stimulating a cyclic immigration of immune cells, leading to islet inflammation, termed insulitis.[19] Because of their key roles in disease initiation and progression, these immune cells and signaling molecules have been the primary focus of modulation for the majority of T1DM therapeutics aimed at blocking and/or reversing insulitis. Some of these drugs are designed to correct the imbalance of Th1 and Th2 T-helper cells, as this has been a long-identified hallmark of T1DM.[20] Development of these classes of drugs as T1DM therapeutics has been facilitated by their FDA-approval for use in other diseases, such as emphysema, rheumatoid arthritis, and psoriasis. Despite the potential benefits of these drugs and their positive outlook in Phase II and Phase III clinical trials for T1DM, they are associated with a variety of serious side effects, including renal toxicity, cytokine storms, and increased susceptibility to infection.[21,22] Antibody-targeted therapies are proving to withstand the rigors of testing in animal models and are showing more limited adverse side-effects.

The evolving field of T1DM therapy: Understanding the role of other cell types

The definition of T1DM is evolving with the field, and now recognizes dysfunction in other cell types/tissues besides the immune system. Currently, the only pharmaceutical approved by the U.S. Food and Drug Administration for the adjuvant treatment of T1DM is pramlintide acetate (Symlin®), an amylin analog. Amylin is co-secreted in the insulin granule and, once released, binds to various G protein-coupled receptors, including the calcitonin receptor, and acts to inhibit glucagon secretion and slow gastric emptying time.[23] In this way, amylin targets the dysfunctional glucagon secretion from the alpha-cells of the pancreatic islet characteristic of T1DM. Adverse events reported from trials with pramlintide acetate include hypoglycemia, nausea, vomiting, and anorexia and were most commonly noted during the first month of therapy.[24] Amylin is primarily used in T1DM individuals who show poor post-prandial glucose control, which makes sense biologically, as glucagon is the signal for the fasting state. Therefore, inappropriate secretion of glucagon after eating actively contributes to hyperglycemia.

With synthetic amylin and insulin being the only FDA-approved drugs for T1DM therapy, and the inadequate glycemic control many individuals with T1DM are able to achieve, there exists a wide space for discovery and validation of other classes of T1DM therapeutics. We performed a systematic review of the current clinical trials focused on T1DM and the mechanisms of action of the therapeutics being explored. Of the 2090 registered "Type 1 Diabetes Mellitus" clinical trials on clinicaltrials.gov, 212 were identified to be investigating a drug, or combination of drugs, meant to improve T1DM standard of care as an adjuvant therapy to insulin. All studies were counted regardless of status with the exception of those that had been withdrawn. The remaining 1878 studies focused on insulin formulation, transplantation, stem cells, specific comorbidities and events (i.e. hypoglycemic episodes, ketoacidosis, exercise, gastroparesis, diabetic nephropathy, muscolo-skeletal abnormalities), or mental health and behavioral interventions, which will not be discussed further in this review. Of the 212 drug trials selected, 42% were

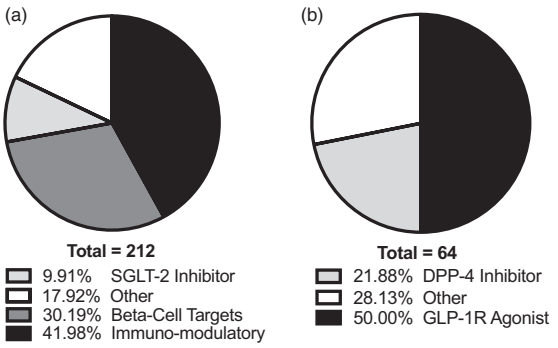

Figure 1. Targets of T1D therapeutics currently in clinical trials are focused primarily on inflammatory and GLP1R pathway modulation. Stratification of clinical trials for T1D therapeutics as of August 2017 shown by system (a) and beta-cell signaling pathway (b).

targeting immune modulators, 30% were targeting the beta-cell directly, 10% were focused on SGLT-2 inhibitors, and the remaining 18% targeted other aspects of disease pathology such as peripheral glucose uptake and/or systemic anti-oxidant status (Figure 1(a)). While it may seem that there is an equal balance on immune-modulatory and beta-cell-centric drug development, 72% of those registered clinical trials specifically targeting the beta-cell were focused on only one signaling pathway: that mediated by the glucagon-like peptide 1 receptor (GLP1R), through dipeptidylpeptidase 4 (DPP-4) inhibition or GLP1R activation (Figure 1(b)).

GLP-1 analogs have been used as type 2 diabetes mellitus (T2DM) therapeutics for the past 12 years, either as standalone drugs or as adjuvants to metformin or insulin. Endogenous GLP-1 is has long been known to be secreted by intestinal L-cells of the gut, where it promotes decreased rates of gastric emptying, improving one of the organ dysfunctions of T2DM.[25] Hypothalamic control of satiety and weight loss, independent of eating behaviors, is also mediated by the action of GLP-1 and further explains the ability of GLP-1 mimetics to modulate T2D pathophysiology.[26] Thus, GLP-1-based therapeutics target multiple cell/organ systems to elicit their modest weight loss effects in individuals with T2DM.[27] Interestingly, though, GLP-1 analogs also referred to as GLP-1 receptor agonists (GLP1-R agonists) were found to elicit robust beta-cell replication and survival in pre-clinical models, resulting in maintenance of beta cell mass, in the context of T2D, while also modulating glucagon levels.[28–30] GLP-1 is an amplifier of glucose stimulated insulin secretion in the beta-cell, and it does so by increasing cyclic adenosine monophosphate (cAMP) production by GLP1R-mediated activation of a stimulatory G protein. The action of GLP-1 requires stimulatory glucose concentrations in order to potentiate the insulin secretion process; therefore, stable GLP-1 mimetics have a very low-risk of hypoglycemia as they promote insulin secretion only in the presence of high levels of blood glucose. The action of gut-produced GLP-1 as hormone that acts on the beta-cell GLP-1 receptor has long been questioned, though, as endogenous GLP-1 undergoes rapid inactivation by DPP-4, resulting in a serum half-life of less than 2 min.[31] Recently, it has been confirmed that the alpha-cells of the pancreatic islet also produce and secrete GLP-1, where it is able to act locally on the beta-cell in a paracrine manner.[27] Because of their stability, though, GLP-1 agonists are able to achieve high circulating levels and do act, in part, as hormones on the beta-cell GLP-1 receptor.[32] These and other findings have fueled investigation of the potential for GLP-1R agonists to be utilized as T1DM adjuvant therapies.

There are a variety of formulations of GLP-1 analogs, allowing for different dosing regimens: exenatide (2×/daily), liraglutide (1×/daily), albiglutide (1×/weekly), and dulaglutide (1×/weekly). Only exenatide and liraglutide have been extensively investigated for use in individuals with T1DM and are currently in phase 4 clinical trials, with promising results. The current state and next moves for the use of GLP-1 analogs for the adjuvant treatment of T1DM have been thoroughly reviewed[33,34]; in brief, addition of a GLP-1 analog to an insulin regimen resulted in improved HbA1c, weight loss, and reduced the total volume of insulin required, and therefore concurrently reduced risk of hypoglycemia. While the therapeutic potential for these drugs is promising, a major concern is patient adherence to therapy, as GLP-1 analogs are administered via subcutaneous injection, up to three times a day. For many individuals with T1DM, adding an additional injectable drug on top of insulin, with a different dosing regimen, might not be well-accepted.

Dipeptidyl peptidase 4 (DPP4) is the endogenous enzyme responsible for the degradation of GLP-1. DPP-4 inhibitors, also widely used in the treatment of T2DM, have been recently tested for their efficacy in improving blood glucose control for patients with T1DM. Despite promising results in pre-clinical mouse models, they have been found to be ineffective at improving blood glucose parameters in T1DM human patients, likely due to the less robust effect DPP4 inhibition has on systemic GLP-1 levels.[35,36] In contrast to stable GLP-1 analogs, which do act in part as hormones on the beta-cell GLP-1 receptor, the effect of DPP-4 inhibitors on blood glucose control in a pre-clinical model does not require the beta-cell GLP-1 receptor,[32] possibly explanatory of their decreased efficacy in human T1DM clinical trials.

The remaining 28% of beta-cell focused T1DM clinical trials evaluated the use of a variety of drugs and natural compounds on the management of glucose parameters. Ten trials investigated Vitamin D[37,38] or polyunsaturated fatty acids (PUFAs),[39] while the remaining eight assessed various drugs, including ER chaperones (TUDCA),[40] glucokinase activators (AZD1656),[41,42] K_{ATP} channel agonists (Diazoxide),[43] calcium ion channel inhibitors (Verapamil),[44] ornithine decarboxylase inhibitors (Difluromethylornithine),[45] PPARγ activators (Plioglitazone),[46] and the novel INGAP peptide.[47] Due to the brevity of this review, these targets will not be discussed here.

Gαz: A possible new T1DM therapeutic target

While the GLP1R agonists are promising as T1DM adjuvant therapies, they are not effective in all patients, sometimes have some intolerable side effects, and are still being

debated about whether they will contribute to beta-cell failure over time.[33] Furthermore, they currently have a black-box warning due to a potential increased risk of pancreatic and thyroid cancer.[48] Therefore, identifying and validating other signaling pathways acting in the beta-cell may yield new therapeutics to replace GLP1R agonists or allow their dose reduction. Our lab has identified a counter-regulatory pathway for the GLP1R in the pancreatic islet. This pathway is mediated by the inhibitory G-protein, G_z. Study of G_z began less than 30 years ago, when it was first identified and shown to have a pertussis toxin-insensitive alpha subunit ($G\alpha_z$), making it a unique among the inhibitory G protein subfamily.[49,50] Additional differentiating aspects of $G\alpha_z$ have been reviewed previously and include its extremely slow intrinsic GTP hydrolysis rate, distinct downstream signaling molecules, and limited tissue distribution.[51] Importantly, $G\alpha_z$ is expressed in pancreatic islets and interacts with adenylate cyclase to inhibit production of cyclic adenosine monophosphate (cAMP).[52,53] In addition to this canonical inhibitory G protein role, $G\alpha_z$ also binds to Rap1 GTPase-activating protein (Rap1GAP) and recruits it to the plasma membrane, where it can inhibit the monomeric Ras-family G protein, Rap1.[52]

A role of $G\alpha_z$ in beta-cell function was first established in 2005, where it was found that prostaglandin E1 (PGE1) regulated glucose-stimulated insulin secretion from the Ins-1 (832/13) beta-cell line in a pertussis-toxin independent manner, implicating G_z as coupling to the receptor for PGE1.[54] This was confirmed by protein knock-down and overexpression of an inhibitor of $G\alpha_z$.[54] Balb/c mice lacking $G\alpha_z$ showed increased insulin secretion after glucose challenge, a corresponding decrease in blood glucose levels, and more robust glucose-stimulated insulin secretion from isolated islets.[55] Finally, $G\alpha_z$-null C57BL/6N mice were protected from T2DM-like pathophysiology when subjected to high-fat diet feeding.[56] To determine if $G\alpha_z$ was a key player in the development and/or progression of T1DM, wild-type and $G\alpha_z$-null C57BL/6N animals were subjected to a multiple low-dose streptozotocin (STZ) regimen to induce T1DM-like beta-cell death and hyperglycemia. A sub-therapeutic dose of the stable GLP-1 analog, exendin-4, was also used in cohorts of mice. $G\alpha_z$-null mice simultaneously treated with exendin-4 maintained near euglycemia following STZ administration.[57] Additionally, loss of $G\alpha_z$ resulted in maintenance of beta-cell fractional area, explained by both increased beta-cell replication and decreased beta-cell death.[57] Loss of $G\alpha_z$ was also investigated in the non-obese diabetic (NOD) mouse model, a more physiologically relevant model of the insulitis observed in the human disease. At 36 weeks of age, when 80% of wild-type NOD animals had become diabetic, $G\alpha_z$-null NOD mice were entirely protected from developing hyperglycemia.[58] The ability of the $G\alpha_z$-null NOD mouse to maintain euglycemia was not only explained by increased beta-cell fractional area (again with increased beta-cell replication and decreased beta-cell death), but also a progressive decline in immune infiltration of the islet.[58] Of importance, wild-type expression of $G\alpha_z$ was confirmed to be negligible in both CD4+ and CD8+ splenic T helper cells, and did not appear to alter type 1 T helper cell response.[58] These results were the first to demonstrate that loss of $G\alpha_z$ improved T1DM outcomes as related to beta-cell function, survival, and replication, but also promoted a less inflammatory islet milieu. Overall, we believe this pathway and its downstream targets may provide a new, multi-faceted type of target in the T1DM therapeutic space.

It may also be possible to modulate this inhibitory beta-cell pathway through its upstream signaling pathway. Genetically obese C57BL/6J Leptin$^{Ob/Ob}$ mice have severe insulin resistance and T2DM-like fasting hyperglycemia and glucose intolerance. Islets from C57BL/6J$^{Ob/Ob}$ mice respond strongly to the selective E prostanoid receptor 3 (EP_3) agonist, sulprostone, to reduce glucose-stimulated insulin secretion in a pertussis toxin-insensitive manner.[56] The primary endogenous ligand for EP_3, prostaglandin E_2 (PGE_2), is derived from the omega-6 polyunsaturated fatty acid, arachidonic acid (AA), a component of plasma membrane phospholipids. Eicosapentaenoic acid (EPA) is an omega-3 polyunsaturated fatty acid that can displace AA from plasma membrane phospholipids. Its metabolism yields PGE_3. PGE_2 is 10 times more potent at inhibiting glucose-stimulated insulin secretion than PGE_3.[59] Enriching islets with EPA, either *ex vivo* or through a dietary intervention, results in decreased PGE_2 production in favor of PGE_3.[59] Wild-type NOD mice fed an EPA-enriched diet show increased *in vivo* and *ex vivo* beta-cell function, suggesting that a dietary intervention might impact the counter-regulatory pathways stimulating $G\alpha_z$ in the beta-cell.[59] Yet, dietary interventions are complicated by numerous parameters, and a more complete understanding of the PGE_2-EP_3-$G\alpha_z$ pathway is crucial to moving toward development of potential therapeutics and is the focus of current investigation.

In sum, $G\alpha_z$ and its upstream and downstream signaling pathways may be ideal targets for the development of

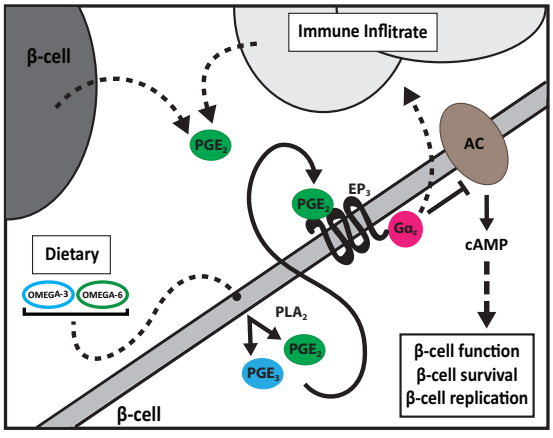

Figure 2. Signaling by activated beta-cell $G\alpha_z$ inhibits beta-cell health in the context of T1D. $G\alpha_z$ is a tonic regulator of cAMP production through its relationship with the GPCR, EP_3. When present, PGE_2 binds to EP_3, resulting in activation of $G\alpha_z$. PGE_2 production is modulated by dietary polyunsaturated fatty acid consumption. When $G\alpha_z$ is inhibited, by modulation of PGE_2 production, or directly at the level of the G-protein, in the context of T1D pathophysiology, the result increased beta cell function, survival, and replication, and coincidental inhibition of immune infiltration. (A color version of this figure is available in the online journal.)

novel T1DM therapies (Figure 2). The tissue distribution of $G\alpha_z$ is quite limited, and loss of $G\alpha_z$ modulates both beta-cell parameters (function, survival, and proliferation), as well as the immune response. Through small molecule targeting or dietary manipulation, inhibition of $G\alpha_z$ might be able to halt the early development of T1DM, while individuals still have significant functional beta-cell mass. Furthermore, such drugs could be used as adjuvant therapies to insulin and/or GLP-1 analogs.

Conclusions

Until recently, the field of non-insulin T1DM therapeutics has primarily been focused on areas of study that have proven successful for other autoimmune diseases. More recent therapeutics already being used clinically or in clinical trials address other primary deficiencies besides the immune system. These include amylin and the GLP-1 analogs. While there are certainly benefits of investigating these drugs as T1DM therapeutic adjuvants, they also pose a host of negative side effects, have limited efficacy, and tend to improve only one facet of the disease pathology. Therefore, there is a particular need for novel therapeutic targets to be validated, and exploring the signaling pathway mediated by $G\alpha_z$, which affects not only beta-cell function but also the immune response, is timely and relevant to the current state of the field.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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