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Type 1 Diabetes Prevention and Treatment Utilizing Gene and Cell Replacement Therapy

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A B S T R A C T

Recent developments in molecular and cellular biology hold promise for creating innovative approaches to treat and potentially cure type 1 diabetes. Specifically, the restoration of insulin secretion through gene therapy or cell replacement techniques is now a conceivable goal. However, the complexity of the β- cell must be acknowledged, as many characteristics of this highly specialized secretory cell need to be accurately replicated in alternative cell types. Insulin secretion is typically regulated and occurs rapidly in response to the metabolic demands of the body, particularly in relation to fluctuations in blood glucose levels. This regulated secretion is crucial to prevent both hyperglycemic and hypoglycemic episodes and relies on the capacity of cells to store insulin in secretory granules, which are released through exocytosis in response to physiological cues. Additionally, any newly engineered insulin-secreting cells must be capable of adjusting to changes in insulin needs that arise from factors such as physical activity, body weight fluctuations, and aging. Long-term regulation of insulin secretion is also vital to prevent "clinical shifting," which may result from excessive insulin production, leading to increased fat accumulation and cardiovascular issues. Lastly, it is essential to ensure that any newly created or transplanted surrogate β-cells are safeguarded against detection by the immune system, particularly to prevent autoimmune destruction.

Keywords: Diabetes, Type-1 Diabetes, Gene, Cell replacement therapy, β- cell, Gene therapy, Insulin. GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; TGN, trans-Golgi network.

INTRODUCTION

Recent advancements in the reversal of type 1 diabetes through islet transplantation, as highlighted by the Edmonton group, have fostered a renewed sense of hope within the medical community. Nevertheless, it is evident that human islets will never be produced in quantities sufficient to meet the global demand of all diabetes patients. Additionally, islet transplantation necessitates long-term, and likely lifelong, immunosuppressive therapy. A compelling strategy to address both of these challenges could involve gene therapy or cell-replacement therapy, which are two potential methods for restoring endogenous insulin production that we will elaborate on below. The aim of this article is to evaluate these two techniques within an objective framework and to establish minimal criteria that we believe must be fulfilled for their clinical applicability. Our intention is not to provide a comprehensive review of the field, nor to advocate for any particular viewpoint on the issues at hand. Our motivation is clear and deeply felt. We are particularly concerned about the growing number of publications, frequently appearing in esteemed and widely circulated journals, that assert progress towards a diabetes cure through gene or cell therapy. Such publications can be misleading, and the individuals most affected—primarily diabetes patients and their families—are often those who are misled, especially given the attention these studies receive in mainstream media. We urge all stakeholders to approach research in this domain with a discerning perspective; it is our aspiration that this article will contribute positively to that endeavor. However, we do not intend to imply that these methods are destined for failure; rather, we hope that the guidelines presented herein will facilitate the safe and effective implementation of these therapies for patients.

Gene Therapy

Insulin gene therapy encompasses any method that entails the introduction of an exogenous gene into various cell types within the body, enabling these cells to synthesize insulin. The introduced gene may be the insulin gene itself, potentially regulated by a tissue-specific promoter to facilitate expression in a targeted non-β-cell type, or it may involve a gene that encodes a factor capable of activating the insulin gene, thus promoting insulin production in non-traditional cells. Additionally, this definition of gene therapy includes the process of inducing stem-cell differentiation into β-cells or cells exhibiting an insulin-producing phenotype through molecular interventions within the patient.

Cell-Replacement Therapy

The term "cell-replacement therapy" refers to the implantation of surrogate β-cells and encompasses all techniques aimed at generating or expanding insulin-producing cells in vitro, followed by their implantation (or reimplantation if derived from the same individual) into the patient. These cells may originate from β-cell sources, potentially being conditionally immortalized to facilitate unlimited growth in culture, or they may be non-β-cells that have been engineered to produce insulin. Alternatively, the cells could be derived from stem cells, whether adult or embryonic, that have been prompted to differentiate into β-cells (or selected for this purpose) in vitro.

Historical Background

The expression of the insulin gene in non-β-cells is not a novel concept. In fact, the first documented instance occurred in 1983 [2], when insulin was expressed in At T20 cells, which are mouse pituitary corticotrophs. This pioneering experiment demonstrated the feasibility of synthesizing pro-insulin in a non-β-cell environment, where it could be appropriately sorted into secretory granules and converted into insulin, provided that the cell type was a professional, regulated secretory cell. It suggested that various regulated secretory cell types could manage an ectopically expressed hormone precursor and release the fully processed hormone in a controlled manner. These findings contributed to a significant review by Regis Kelly [3] in 1985, which outlined the key characteristics of both constitutive and regulated secretory pathways. The introduction of genetically engineered surrogate β-cells into diabetic animals, particularly those induced by streptozotocin, is also not particularly groundbreaking. The earliest accounts of this practice date back to 1987, when Richard Selden [4] and colleagues reported the implantation of fibroblasts expressing pro-insulin, driven by the metallothionein promoter, into diabetic mice. Unsurprisingly, the mice succumbed to hypoglycemia due to the excessive and unregulated release of (pro)insulin from the implanted fibroblasts. Notably, 1983 also marked the initial attempt at insulin gene therapy, with reports of insulin expression in vivo in animals following the injection of liposome-encapsulated insulin cDNA [5]. Since these initial insulin gene therapy experiments, numerous additional studies have been published.

Insulin is typically released in a constitutive manner, as regulated secretory cells have not been utilized in many studies, including those by Chen *et al*., [6] and Lee *et al*., [7] Numerous comprehensive reviews have addressed the overarching topic of gene therapy for diabetes. However, a recent investigation demonstrating glucose-regulated insulin secretion from a genetically modified intestinal K-cell line stands out as a notable exception, as it employs authentic regulated secretory cells. Given the current state of research in this field, it is disheartening to acknowledge that significant progress has not been made over the past 18 years; the same concepts have been revisited repeatedly. A key contextual shift has occurred: the pursuit of a "cure for diabetes" [8-11], which has always been a commendable academic objective, has now transformed into an industrial ambition with considerable stakes. This shift has resulted in heightened media attention on even the most rudimentary studies, yet the general public—and occasionally our peers—struggle to differentiate between exaggerated claims and genuine advancements.

Standard Observations

What do we anticipate from gene therapy or cell replacement therapy as a treatment (or, preferably, as we call it, a "cure") for type 1 diabetes? First and foremost, the intervention—regardless of its type—must cause lower rates of morbidity and mortality than the illness. For instance, based on these straightforward standards, it is unacceptable to subject a young child to immunosuppression for the rest of their life in order to "cure" diabetes by transplantation. Even though these safety and ethical concerns are extremely important, we are not the right people to talk about them here, and this is not the right forum. Even though these safety and ethical concerns are extremely important, we are not the right people to talk about them here, and this is not the right forum. It is sufficient to state that, given the paucity of clinical data, we should all be mindful of the potential risks associated with gene and cell therapy and take a sufficiently modest and cautious stance. On the other hand, we ought to be able to specify exactly what we anticipate from such a treatment in terms of the metabolic result. The results of intensive insulin therapy, whether administered by repeated insulin injections or an externally regulated (open-loop) insulin pump, are typically regarded as satisfactory by both patients and doctors. Insulin therapy is becoming more efficient and "patient friendly" thanks to recent advancements in insulin

formulations and equipment. However, this type of therapy is not flawless. Diurnal glycemic excursions are undoubtedly more severe than in nondiabetic people with second-by-second regulation of insulin secretion, and lowering hyperglycemia to normoglycemia is invariably linked to an increased risk of hypoglycemia. Patients must regularly check their blood sugar levels, and the injections (or pump use) itself present challenges.

Despite this, most people would agree that the ongoing development of new insulin analogs, along with better and more "patient friendly" monitoring and delivery systems, makes the current "standard" therapy rather good. Indeed, every creative therapeutic technique faces a significant competitive threat from such therapy. In the sections that follow, we try to explain how effectively the β-cell regulates blood sugar levels and what features of β-cell function are essential for a non-β-cell to achieve normal metabolic control (i.e., how intelligent and considerate will scientists need to be?). It is evident that when addressing an autoimmune disease like type 1 diabetes, the more closely we replicate a β--cell's functional details, the more probable it is that the newly generated cell will be destroyed by the autoimmune system, just as the host's natural β-cells were when the disease first started. Nonetheless, the immunological properties of cells can be altered through gene therapy and cell replacement therapy, making them less vulnerable to autoimmune destruction or rejection following implantation. These problems will also be addressed.

β-cells in Glycemia Controllation

This inquiry has a very clear answer: very nice indeed. No reasonable scientist would choose the β-cell as the first cell to be replaced by molecular engineering technologies! In fact, developing the ideal surrogate β-cell might out to be too difficult for our current level of understanding and equipment. The β-cell brings together a number of noteworthy characteristics into a single functional unit, including regulated pro-insulin transcription and translation, a regulated secretory pathway with all of its unique characteristics, and stimulus-secretion coupling (with exquisite sensitivity, most notably but not exclusively to glucose). When combined, these characteristics make the β-cell exceptionally well-suited to its physiological function. We are growing more conscious of the combination power of particular genes, proteins, or metabolic pathways in this post-genomic era. Therefore, the β-cell force also resides in a special combination of functions, rather than each function being exclusive to this cell type. Think about two instances. The non-limiting rates of glucose transport into the cell and glucose phosphorylation, mostly by the high Km enzyme glucokinase, are similar in hepatocytes and β-cells. They are therefore well suited to react with suitable modifications in glycolytic flow to distinct changes in ambient glucose. Even while some hepatoma lines may have a controlled secretory pathway, this characteristic by itself will not enable the hepatocyte to become a reliable β-cell surrogate by simply expressing the insulin gene [13]. Similar to this, some endocrine cells and cell lines may not have the stimulus-secretion machinery necessary to release insulin in response to glucose, but they do have the controlled secretory pathway and the capacity to store fully processed insulin in secretory granules.

The β-cell Combined Stimulus Secretion Connecting to a System

In vivo, the β-cell continuously monitors metabolic status and can respond to even the smallest changes in the concentration of various metabolites, most notably glucose, by producing the proper secondary stimulus-coupling signals. This is combined with regulatory input from neuropeptides and other signaling pathways. These signals affect other (equally important) β-cell processes, such as pro-insulin synthesis and conversion, insulin breakdown, and β-cell growth and survival, in addition to the well-known changes in insulin production.

Secretagogues for Glucose and Additional Substrates

It is important to emphasize several unique metabolic characteristics of the β-cell that allow for the generation of secondary stimulus-coupling signals without delving too deeply into the molecular details. Because the β-cell expresses both glucokinase and the “high Km” glucose transporter (GLUT2 or another), it can monitor circulating glucose concentrations in the physiologically relevant range of 2–20 mmol/l [14]. Although this is a rather uncommon characteristic of mammalian cells, hepatocytes, certain gut cells, and hypothalamus neurons also carry these components, allowing them to sense extracellular glucose. Very low levels of lactate dehydrogenase and plasma membrane monocarboxylate pyruvate/lactate transporter activities are also typical of primary islet β-cell metabolism, but not of other mammalian cell types [15]. As a result, lactate production in initial β-cells is nearly undetectable. In order to re-oxidize cytosolic NADH back to NAD+, which is necessary for glycolysis and is typically supplied by lactate dehydrogenase in the majority of eukaryotic cells, there is a noticeable increase in mitochondrial metabolic shuttle activities (such as the glycerol-3-phosphate shuttle). To effectively direct pyruvate, the primary product of glycolysis in the absence of lactate production, toward mitochondrial tricarboxylic acid cycle and oxidative phosphorylation metabolism for efficient ATP production, the primary islet β-cell also possesses several times more pyruvate carboxylase activity [16].

One important metabolic stimulus-coupling factor in the β-cell that regulates insulin release is changes in intracellular ATP generation (17). Because of the uniquely elevated pyruvate carboxylase activity in β-cells, glucose-regulated anaplerosis can produce more potential metabolic stimulus-coupling signals, including glutamate and malonyl-CoA, which are produced from the tricarboxylic acid cycle. Thus, in addition to ensuring the energy needed for its regular daily activities, the β-cell possesses a well balanced set of metabolic enzymes that are ready to produce metabolic secondary signals to control its activity, specifically control of insulin exocytosis. This distinguishes mammalian cells from others. Therefore, simply adding the insulin gene and the glucose sensing capabilities of GLUT2 and glucokinase to cells with a regulated secretory pathway will not be sufficient to create surrogate β-cells with normal or at least adequate glucose stimulus-secretion coupling capacity. It is necessary to take into account the balance between the expression and activity of other downstream metabolic enzymes. In this context, it is noteworthy that, contrary to what may have been naively expected, expression of GLUT2 and glucokinase in primary intermediate pituitary cells transgenically engineered to produce insulin resulted in impaired glucose metabolism, glucose toxicity, and apoptosis (18). For other fuel secretagogues, like leucine, to have an effect on secretion, they must also undergo metabolism. Common signals are produced as a result of the different metabolites produced in this manner "plugging in" to the metabolic circuitry previously mentioned.

Secretion of Insulin Management, including Neuroendocrine Peptides

Other significant β-cell function regulators should be regarded as specific to this cell type. The first is the so-called incretin effect, which is a communication between the endocrine pancreas and the gut that supports the secretion and synthesis of insulin that is regulated by nutrients. The term "incretin" describes the peptide hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), also referred to as gastric inhibitory polypeptide [19].

Because GLP-1 receptors are mostly located on β-cells (and certain hypothalamic neurons), insulin secretion, pro-insulin production, and β-cell proliferation are all specifically regulated by glucose-regulated GLP-1 [20]. Therefore, in addition to and supplementary to nutrient-induced metabolic control, GLP-1 is another unique regulator of β-cell function. When creating surrogate β-cells, the GLP-1 receptor's presence would be crucial in order to obtain a complete postprandial response. Additionally, β-cell activity, particularly insulin secretion, can be influenced by neural input. This is especially crucial when metabolic stress is present [21]. β-cell replacement therapy would almost certainly result in the loss of such neuronal input.

Subsequent Effectors that Overlap

To ensure greater exocytosis, all stimulus-secretion coupling pathways must eventually converge at a single location. Therefore, there aren't many second messengers or coupling factors that are the most distal (such as Ca2+ and cAMP), and the majority are most likely ubiquitous in their function in all regulated secretory cell types. Each type of cell, however, has its own methods for regulating their levels. Moreover, the process via which elevated exocytosis results from modifications in effector/messenger levels may also be cell-specific. Take a look at these instances. In many (perhaps all) regulated cell types, an increase in intracellular (free) Ca2+ ([Ca2+]i) concentration can trigger exocytosis on its own. However, the KATP-channel (the target of sulfonylureas) is necessary for a key pathway in the β-cell that leads from glucose stimulation to raised [Ca2+]i and hence enhanced insulin production.

PHHI (persistent hyperinsulinemichypoglycemia of infancy) is a collective term for illness conditions and uncontrolled insulin secretion caused by mutations in the KATP-channel gene in humans [22]. Additionally, there is strong evidence for a significant glucose-signaling pathway that is independent of the KATP-channel but still requires increased cytosolic Ca2+ [23] and other variables [24]. Although cAMP can probably increase granule exocytosis in most endocrine cells, the manner it intensifies glucose-stimulated secretion might be due to β-cell-specific mechanisms.

The ability of glucose to modify the stimulation of insulin secretion by GLP-1 is based on this specific aspect of stimulus-secretion coupling. Lastly, the molecular process of exocytosis itself has just recently begun to be understood, and further research is needed to determine how this phenomenon is finally controlled. It's possible that elements of this final stage of insulin secretion are specific to the β-cell. Even if not everything is known yet, the intricacy of the β-cell stimulus-secretion relationship is already apparent. While some of the system's characteristics are specific to this cell, others could manifest in different ways. Surrogate β-cells will probably not have properly controlled insulin production if only one or two parts or components are expressed in a semi-random manner. This could also be harmful to cell survival.

One of the biggest challenges is still figuring out how to reverse diabetes with a minimal amount of regulation that is tolerable and free of undesirable side effects (see below).

(Pro) Insulin Manufacturing: Maintaining Secretion

Control of the manufacture of Insulin

Numerous transcription factors, some of which interact with one another, are involved in the expression of the insulin gene [25]. Moreover, preproinsulin mRNA stability, which is probably β-cell-specific, and hetero-nuclear mRNA processing are also necessary for sustaining preproinsulin mRNA levels in addition to gene promoter activity. The intracellular reserves of insulin can be kept reasonably constant by the pancreatic β-cell. Pro-insulin production is rapidly upregulated at the translational level in response to the loss of insulin from the β-cell due to glucose-induced exocytosis [26, 27]. Under typical physiological conditions, this is the main way to control the β- cell's generation of pro-insulin. Nevertheless, over time, transcriptional regulation and insulin breakdown within the β-cell complement this translational control. With distinct signals interacting with cis elements in the untranslated regions of preproinsulin mRNA, the β-cell appears to be the only cell that can specifically regulate the translation of pro-insulin production in response to glucose [28]. Although preproinsulin cDNA composition for gene therapy vectors must be carefully considered, it might not be enough on its own to enable translational control in a surrogate β-cell.

Pro-insulin swapping to generate Insulin

Two endoproteases, PC1 (also called PC3) and PC2, along with an exopeptidase called carboxypeptidase-H, mediate the conversion of pro-insulin [29, 30]. After secretory granules have become acidified and in the presence of the high intra-granular [Ca2+] that is currently present, conversion occurs within them. Furthermore, a comparable specific translational control of the manufacture of PC1 and PC2 parallels the glucose-induced rise in pro insulin biosynthesis. As long as the pro insulin molecule's dibasic processing sites are modified to allow the generic pro-protein convertase furin to recognize it, PC1 and PC2 are not necessarily necessary for in vivo pro-insulin conversion in other mammalian cell types [31]. Even though pro insulin may be released by the constitutive pathway (see below), it is digested in these situations. Nevertheless, conversion in these artificial conditions is usually less effective than in the granules of the β-cell-regulated secretory pathway's natural environment. Moreover, pro insulin may become immunogenic due to the mutations required to make it susceptible to furin cleavage.

Insulin Development Processes

Within a minute of exposing the β-cell to a secretagogues, insulin secretion can be triggered. This is the distinguishing feature of the controlled secretory route that involves the exocytosis of insulin that is stored in secretory granules. The off-reaction is as quick. These extraordinarily quick kinetics of insulin secretion are essential for maintaining normal glucose homeostasis. The regulation of exocytosis is made possible by stimulus-secretion coupling, which we have already covered. However, a series of highly specialized events occur close to this, which collectively enable the proper sorting of pro insulin to nascent granules in the trans-Golgi network (TGN) and the development of functional competence in granules [32, 33].

However, very little is known about these occurrences. Until further required components are identified, it is unlikely that the controlled pathway can be reconstituted in a non-specialized cell. However, in rat β-cells (and, it is assumed, human cells as well), it has been demonstrated that almost 99 percent of all freshly generated pro insulin molecules are directed to developing secretory granules and are therefore managed by the controlled secretory route. It has undoubtedly long been understood that controlled insulin secretion cannot be achieved by just expressing pro insulin, even when combined with the conversion enzymes PC1 and PC2. It's unclear how many extra "regulated pathway" genes would have to be expressed.

Essential or Controlled Secretion

What occurs if the controlled route is not used to secrete insulin? It is believed that the so-called constitutive route allows all cells to release proteins. This pathway enables vesicles to move from the TGN to the membrane of the plasma cell in about 20 minutes, after which they undergo instantaneous and uncontrollable exocytosis [3]. The only ready-made way to regulate is at the synthesis level. Could type 1 diabetes ever be reversed by cells releasing insulin through the constitutive mechanism, but with the most advanced regulation conceivable at the level of gene transcription? It appears that many people think so [see, for instance, [6, 7, 34-36]. We don't. Let's look at the facts. It takes hours, not minutes, to stimulate transcription in a quantitatively meaningful way, as even the most hopeful calculations show. Furthermore, the minimum time required from stimulating transcription to the secretion of the first newly formed protein molecules via the constitutive pathway is increased by at least 20 minutes because we are not

dealing with a cytosolic protein but rather a precursor (preproinsulin) that must pass through the secretory pathway prior to exocytosis. It is reasonable to assume that a minimum of two hours must pass between exposing a cell to a transcriptional stimulus and the release of the first pro insulin or insulin molecules produced as a result of that stimulation. Experiments in animals support this estimate [7].

Even worse, unless the mRNA has an exceptionally short half-life—which is not the case with preproinsulin mRNA—the "off" response for transcriptional regulation is invariably slow. Although the half-life of preproinsulin mRNA is shortened (about 6 hours) when it is expressed in hepatocytes as opposed to the β-cell (>24 hours) [28], this period of time is still much longer than the few minutes needed to stop insulin secretion once the stimulus is removed. As previously stated, the β-cell typically reserves the regulation of insulin gene transcription for longer-term, adaptive control. When pro-insulin is expressed in constitutive surrogate cells, would it not be more sensible to try to regulate it at the translational level? Here, too, we see issues. First, it is still unclear exactly how translational regulation of pro-insulin production works. Second, achieving the same degree of translational control as the β-cell would not result in kinetics that are equivalent to those of controlled exocytosis.

The lag time for glucose-induced pro-insulin production is 20 minutes, and it peaks at 60 minutes. Within 90 minutes of removing a stimulus, pro-insulin production resumes its baseline rate. Additionally, these figures pertain to synthesis in general rather than secretion, which would extend the time frame by additional valuable minutes even through the constitutive mechanism. All that remains is our initial hypothesis: the β-cell is genuinely intelligent. It features an extremely complex stimulus-secretion coupling mechanism that is specifically designed to modify insulin secretion second by second in response to the individual's metabolic requirements. This is coupled with the controlled secretory pathway, which enables the replenishment of insulin storage after the nearly instantaneous release of precisely the required quantity of stored insulin, regardless of the rate of synthesis.

Advantage of the Physiological and Clinical Aspects Regarding Insulin Release

It might be challenging to achieve strict metabolic control with insulin regimens that involve insulin pump therapy or numerous daily injections. Exogenous insulin must be administered in a way that replicates both basal and stimulated secretion, two essential elements of endogenous insulin release, in order to accomplish this goal. This fact has been substantiated by the use of insulin pumps in the treatment of type 1 diabetes, where it is well recognized that the remarkable variability in glucose levels observed in our patients cannot be controlled by continuous infusion of insulin at basal levels. Even though most people still struggle to achieve rigorous metabolic regulation, it is becoming increasingly possible because to the development of several novel insulin analogues. In order to bring glucose levels into line with those of healthy patients, genetically modified insulin-producing cells must be able to release insulin when needed in addition to delivering basal insulin if they are to be competitive in the field of diabetes treatment. They must do it without the dangers that come with exogenous insulin replacement in order to be genuinely beneficial—a very difficult task.

The Insulin Secretion Compared: Stimulated or Basal

It is definitely possible to reach basal insulin release. However, this is probably going to happen mostly through the constitutive secretory pathway in many cell types that lack the necessary cellular machinery. It is crucial that basal insulin secretion be modulated as it does in healthy human subjects, even if this release may be relatively steady at any one time. At first look, this might not seem like a crucial requirement because it is only basal. This aspect of insulin release must, however, be flexible since regular life activities like exercise, infection, and weight gain all call for suitable and sometimes quick adjustments in insulin production. Failing to do so may lead to either hyperglycemia or hypoglycemia. Even while these occurrences are not unusual for insulin users at the moment, it would not be ethical to have gene or cell therapy treat them again.

How about insulin secretion that is stimulated? In order for the release of insulin in response to dietary intake to be physiologically appropriate, it must be able to react to both glucose and the building blocks of proteins and lipids [37]. Nevertheless, it cannot be assumed that all the jigsaw pieces are presently in position if such an accomplishment were to be made. It is evident that the gastrointestinal system contributes to the insulin response to oral consumption [38] and that insulin sensitivity [39], in turn, modulates this response. Therefore, if adequate amounts of this life-dependent peptide are to be generated during the physiological state of eating, the incretins, such as GLP-1 [40] and GIP [38], must be able to boost the gain of the insulin-producing cell. Even though we might not fully comprehend all of the β--cell's stimulus-secretion coupling mechanisms just yet, there is a good chance that a system that can react to as-yet-unknown modulators will be produced by building a cell with the entire complement of the known essential sensing and releasing apparatus. Although this is an ambitious objective, it is absolutely essential if we are to cover all the bases.

Insulin responsiveness, body weight, and physical activity

It is evident that both short-term and long-term modulation of β-cell function is possible. Body weight, a scourge of our modern culture, and exercise are maybe two of the best examples. Exercise affects metabolism in both the short and long term. If hypoglycemia is to be prevented, a quick shift in insulin production must counteract the abruptly increased glucose utilization by the insulin-sensitive tissues that occurs after a single bout of aerobic exercise. The physiological alterations brought about by consistent exercise encompass both the short-term training effect and the longer-term effects of each acute exercise session. Because of the training impact, which includes an increase in insulin sensitivity and a corresponding shift in insulin secretion, glucose tolerance often stays almost the same as it was before to starting regular exercise. On the other hand, if glucose tolerance is to stay the same, increases in insulin production are necessary when body weight increases or when body fat compartments are moved to a more central place. The decrease in insulin sensitivity that is usually linked to increases in adiposity must be the cause of this increase in insulin release.

It is still unclear, though, how changes in insulin sensitivity, such as those brought on by exercise and weight fluctuations, affect the β-cell's responsiveness so accurately. In contrast to changes in β-cell mass, adaptation through modification of insulin secretion (or the secretory response) is examined below. It will be crucial to permit output to be increased more than twenty-fold in certain insulin-resistant animals in order to modulate insulin release [39]. Specifically, we require at least partial responses to a few of the following queries. The first question is whether the central nervous system or humoral factors like glucose or free fatty acids that directly affect the insulin-secreting cell mediate the islet's β-cell gain. Second, is the signal from the periphery to the brain humoral or neural if the central nervous system is important? Third, is neural regulation of the islet absolutely necessary if the central nervous system is involved in any part of this process? In the absence of trustworthy responses to some or all of these queries, it might be difficult to confirm that the replicated β-cell has the right sensor or sensors, which could make it more difficult to restore normal glucose homeostasis.

Insulin resistance and ageism.

Improving quality of life must be a key component of a radical shift in our current approach to insulin therapy. An improvement in glucose control must undoubtedly be the other objective, as this should lessen the devastating consequences of the illness and offer hope for a longer lifespan. However, because the designed cell must now deal with the regular physiology of aging, which is linked to an intriguing shift in the control of glucose metabolism, extending life may also result in additional physiological challenges. Healthy aging is linked to a steady decline in glucose tolerance for reasons that are currently unclear. Accordingly, oral glucose tolerance testing indicates that many older people have impaired glucose tolerance or even outright diabetes. Despite the fact that insulin resistance also occurs with aging, this rise in glucose levels seems to be a compensatory reaction to some as-yet-unidentified physiological demand of the aging process [41] and is accomplished by a decrease in insulin release [42]. Therefore, we might need to devise methods that will enable the altered cell to respond to insulin resistance by producing more insulin as usual, which is necessary for younger people, but later in life, the same cell might be able to change its insulin production in the opposite direction, even though insulin resistance is present! Since it is unclear whether the slight decline in glucose tolerance that occurs with healthy aging is necessary to achieve objectives like guaranteeing adequate glucose delivery to tissues like the brain, which use glucose independently of insulin, it is regrettably difficult to determine whether such an adaptation is absolutely necessary. As a "reward" for extending life, we might be putting ourselves and the constructed system through yet another challenge. If so, it will be crucial to determine if modifying insulin secretion alone will be sufficient to overcome this specific obstacle or if it will also require modifying β-cell mass as indicated below.

β-cell mass and physiological insulin release as a physiological adaptive response

It's evident that the mass of β-cells in islets makes up a complicated microorganism that regulates the body's supply and elimination of substrates, with glucose being the most well-researched and easily measured. Given that the β-cell plays a crucial role in maintaining metabolic circumstances that are orientated toward survival, its intricacy is most likely justified. Even if the mechanisms underlying this are undoubtedly not fully understood, it is imperative that we strictly adhere to the necessity of a physiologically responsive system when building cell-based treatment approaches. If the cell is unable to do so, there is a slim chance that systemic redundancies will enable proper compensation to take place in a cell that is not otherwise damaged by the immunological and metabolic problems commonly seen in type 1 diabetes.

A key prerequisite will have been satisfied if the individual modified cell can be made to be physiologically responsive and able to sharply modify its response. However, an adaptive response of a different kind is likely when

longer-term adaptation is required, as is probably the case with insulin-resistant states like obesity. In these conditions, the mass of β-cells is enhanced by both the size of the individual β-cell and the number of β-cells [43, 44]. This increased bulk contributes to a reduction in the secretory burden of individual β-cells. Creating a cell that can change how much insulin it releases will be difficult, but creating a cell that can multiply and only do so under the right conditions will be extremely difficult. It will be significantly more difficult to make sure that any such proliferation is controlled and never results in hypoglycemia or hyperinsulinemia! Removing or destroying some of the implanted surrogate β-cells or replenishing the reservoir in accordance with evolving needs would obviously be an alternative. Until significantly safer methods for delivering genes to patients are devised, such fine-tuning will not be possible in the context of gene-replacement treatment as opposed to cell-replacement therapy. In any case, the challenges facing the scientist and the designed β-cell are significant since secretory demand changes in real life are likely to differ both daily and over an extended period of time.

Is it appropriate to test engineered β-cells' ability to reverse diabetes in humans using animal models?

It is obvious that before this technique is applied to people, tests of the potential of genetically modified, fully responsive "β-cells" to restore glycemic control must be conducted in animals. But it's important to remember the old saying that a rodent is not a person in more ways than one while performing these in vivo evaluations. For good reason, rodent models have been used up to this point in the in vivo research in this field. This could be misleading for some parts of metabolism, such as glucose elimination, but it might be helpful for others. It is now widely acknowledged that at least two mechanisms—insulin-dependent and insulin-independent—are necessary for glucose absorption. While insulin-independent glucose uptake has undoubtedly received less scientific attention, it is just as, if not more, significant in certain situations than insulin-dependent glucose disposal, which is a composite measure of the combination of insulin sensitivity and insulin production [45, 46].

Insulin-independent pathways account for a significant amount of glucose uptake in the brain during the basal state, and basal insulin plays a crucial role in controlling hepatic glucose output [47]. There hasn't been much research done on how these elements interact and how they can potentially balance each other out. Therefore, just when fasting glycemia is almost balanced in one type does not necessarily signify that an experimental strategy incorporating insulin replacement therapy is beneficial in other models. Furthermore, a percentage of glucose absorption into tissues is once more insulin-independent following glucose delivery [45, 46]. The effectiveness of glucose absorption by insulin-independent pathways in animals is typically higher than in people [46], so it can be dangerous to simply extrapolate animal results as the anticipated observation in humans when testing new systems using animal models. The fact that the early stages of insulin release seem to be essential for controlling the glucose excursion following food administration is also significant [48, 49].

Therefore, when a genetically modified system is developed that lacks these essential characteristics for quick response to acute stimulation, it may lead to less enthusiasm when it is transferred from animals to diabetic humans. Naturally, we do not advocate delaying the flow of discovery, even while we do advise exercising some prudence. Instead, we want the methods' design and implementation to be thoroughly tested to make sure they will likely resemble real human physiology before being used on humans.

Clinical Switching

Technology's ability to eliminate the need for exogenous insulin replacement and the hypoglycemia and hyperglycemic fluctuations that come with managing diabetes will undoubtedly be a huge advancement. Naturally, it will also be interesting to see if resolving one clinical issue results in another. While some of these might not have been thought of yet, others can be anticipated. A few instances are relevant because they are illustrative, even though they are not always certain due to the state of the technology.

Exact control of metabolism and corpulence

We have undoubtedly learned a lot from the Diabetes Control and Complications Trial, or DCCT. Actually, the experience is still teaching us a lot. The finding that some trial participants who were randomized to and attained stringent metabolic control acquired significantly more weight than others was one unexpected consequence. It is increasingly clear that these people's phenotypic has undergone "clinical shifting" to encompass characteristics of type 2 diabetes, such as central obesity and an unfavorable lipid profile [50]. Was it possible to avoid this? Most likely not. Nonetheless, it seems that the people who experienced this had a family history of type 2 diabetes, so it might be more predictable going forward. These findings impose the necessary requirement of further adjusting insulin release to

metabolic requirements on the modified β-cell, while also presenting a plethora of additional challenges that will require clinical therapeutic attention.

Cardiovascular disease, pro-insulin, and insulin resistance.

Insulin resistance has been linked to cardiovascular disease. Obesity, especially central adiposity, may undoubtedly be linked to some of this. It would seem wise to try to prevent excessive insulin exposure at this time, if at all feasible, even though there is ongoing discussion about whether insulin resistance is a risk factor and whether hyperinsulinemia is harmful to health. We must therefore attempt to prevent "clinical shifting," which is the transition from a condition of elevated cardiovascular disease risk due to the metabolic disturbance linked to hyperglycemia to a state of elevated cardiovascular disease linked to insulin resistance. Since continuous non-pulsatile insulin treatment is more likely to cause iatrogenic insulin resistance, it may be necessary to use an engineered β-cell that can release insulin in a pulsatile form to prevent this from happening [51, 52]. Parallel to this, giving too much insulin not only increases the danger of hypoglycemia but also causes the insulin receptor to be downregulated, which impairs insulin responsiveness.

Another concern regarding cardiovascular disease is brought up by the clinical experience of pro-insulin as a treatment approach. It has been shown that the insulin precursor molecule effectively lowers glucose in people with type 2 diabetes [53]. Unfortunately, however, a pro-insulin clinical trial had to be stopped due to a potential rise in cardiovascular death. However, it is yet unknown what the actual therapeutic significance of the data from this specific trial is and how pro-insulin may contribute to an increase in mortality. But according to in vitro research, pro-insulin may be able to boost the synthesis of substrates linked to a higher risk of atherogenesis [54]. The release of pro-insulin into plasma may be linked to gene or cell replacement treatment because pro-insulin is a typical secretory product of the human β-cell [55]. If pro-insulin conversion is not as efficient as in the original β-cell, pro-insulin levels may be higher.

Cell Replacement Therapy and the Immune system

Allorejection is one of several variables thought to be responsible for the poor results of the many attempts to transplant human islets over the last 25 years. Insulin production has historically been at best temporary in type 1 diabetic patients having transplanted islets, despite long-term immunosuppression. Modern immunosuppression that stops kidney, heart, or liver Allorejection appears to be ineffective when applied to human islets. At the very least, this issue would be resolved by transduction of the patient's own cells. In fact, auto-transplantation of islets taken from the patient's own pancreas following resection for chronic pancreatitis yields the best islet transplant outcomes [61]. The long-term functionality of auto-transplanted islets raises the possibility that transplanted islets are harmed by existing immunosuppressive treatments. Naturally, the fact that the auto-transplanted islets in these specific clinical situations are not only immune to Allorejection but also immune to the autoimmune onslaught that is the danger of type 1 diabetes complicates the interpretation of these data. Clinical islet transplantation has been severely impeded by the intricacy of managing three distinct immunological attacks: acute rejection, chronic rejection, and disease recurrence. Furthermore, a multitude of in vitro data indicates that cyclosporin, prednisone, or azathioprine may have an impact on β-cell activity.

Even if the initial findings are encouraging, it is yet unknown how the glucocorticoid-free immunosuppressive regimen used in the "Edmonton" protocol may affect β-cell function and survival over the long run [1, 62]. The most significant immunological risk associated with islet transplantation is the last one. As was clearly shown in the case of pancreatic transplantation between monozygotic twins, a number of studies indicate that the loss of function of transplanted islets was due to disease recurrence, even in the face of immunosuppression and HLA matching [63]. Recurrence of GAD65 autoantibodies, a key serological indicator of type 1 diabetes, has been linked to unsuccessful islet transplantation [64]. The gene therapist attempting to generate β-cells, possibly from pancreatic stem cells or reintroduced after extra corporeal transduction and modification, will be haunted by the immune system's amazing recall. There are as many theories and options as there are researchers trying to figure out how to prevent the immune system from attacking the β-cells again. These cells undoubtedly need a barrier to protect them from immunological attack. Creating enclosed islets is not a novel concept. Most people would concur that the state of the art as it is now still doesn't meet the exacting standards needed for clinical use. Hopefully, more study will lead to capsules or synthetic immunological barriers with suitable diffusion properties, sufficient bio-compatibility, and long-term function. It will be crucial to consider the entry of substances like cytokines that affect β-cell function, even though insulin may be able to cross the barrier to assist manage blood glucose. The concept of transducing islets or islet cells with immune-deterrent elements is another approach being used to create genetically modified immunological barriers. Local cytokine or chemokine production at the site of islet implantation and its impact on maintaining long-term β-cell activity are just two of the numerous unknown aspects.

The fundamental processes by which the immune system destroys β-cells have not been thoroughly figured out. Therefore, the hunt for genes encoding components that might render the immune system insensitive to transduced insulin-producing cells or transplanted islets is relegated to trial and error. Confounding factors include the possibility that these studies are only really instructive in the human system and that translations from the NOD mouse or the BB rat to humans will be at best weak and probably unrelated to type 1 diabetes in humans. In fact, it has been demonstrated that the several cures for NOD mice are not very effective in treating type 1 diabetes in humans [65]. Since there isn't a single defective gene that has to be fixed, one could initially assume that gene therapy isn't appropriate for type 1 diabetes. However, it is undoubtedly an alluring idea to molecularly modify transplanted islets or cells to protect them from immune attack in order to prevent Allorejection, disease recurrence, or both. Since the β-cell is unusually sensitive and sensitively regulated, as mentioned above, it will be required to proceed cautiously; the expression of novel genes may result in unfavorable consequences on cell function. If the patient's immune system identifies insulin as a major auto-antigen, the alternative of reintroducing the recipient's cells, such as hepatocytes, following in vitro transduction should avoid the risk of Allorejection, but it does not rule out the possibility of disease recurrence. The fact that up to 60% of children under the age of 10 who are clinically diagnosed with type 1 diabetes may have insulin autoantibodies at the time of diagnosis emphasizes the significance of insulin as a target [66]. Most likely, cytotoxic CD8 T-cells that identify their target by presenting short peptides on the cell surface as class I HLA molecules destroy β-cells. A liver cell transfected with the insulin gene is likely to express class I molecules loaded with insulin peptides because these brief peptides are loaded within the cell. When considering transduction investigations using cells extracted from recipients who have type 1 diabetes, this potential situation must be considered. When using systemic ways of gene transduction to convert cells like hepatocytes into cells that produce insulin, recurrence of the T-cell onslaught must also be taken into account. Additionally, when these elements seem to be in play, it is important to keep in mind that while preclinical studies in the BB rat and NOD mouse may offer proof of principle to appease the stock market, they are less likely to give appropriate preclinical safety measures for subsequent clinical trials.

CONCLUSION

We respond to people who think our expectations are too high by saying that the β-cell and the unique immunological and metabolic limitations of type 1 diabetes, not us, determine the expectations. We believe that having well-regulated insulin secretion will be crucial for treating type 1 diabetes with gene or cell replacement treatment without the need for any additional adjunct insulin therapy. Patients with type 1 diabetes will not benefit from constitutive secretion, regardless of the level of insulin gene transcription regulation that goes along with it. It goes without saying that suitable triggers, such as glucose if at all possible, must further regulate secretion itself. It might be more difficult than previously thought to make ordinarily insensitive cells sensitive to glucose. A thorough evaluation of cell function will be necessary prior to any clinical use. Although the most recent immunosuppressive regimens are less toxic to insulin-secreting cells than more traditional ones, it is to be hoped that greater understanding of the molecular mechanism of both allograft rejection and autoimmune destruction of β-cells will allow for genetic engineering of cells resistant to both. Lastly, it goes without saying that in order to treat type 1 diabetes, new and more abundant sources of cells that secrete insulin will be required. It goes without saying that suitable triggers, such as glucose if at all possible, must further regulate secretion itself. It might be more difficult than previously thought to make ordinarily insensitive cells sensitive to glucose. A thorough evaluation of cell function will be necessary prior to any clinical use. It is hoped that a better understanding of the molecular mechanisms underlying both autoimmune destruction of β-cells and allograft rejection will enable the genetic engineering of cells resistant to both, even though the most recent immunosuppressive regimens are less harmful to insulin-secreting cells than more conventional ones.

Lastly, it goes without saying that in order to treat type 1 diabetes, new and more abundant sources of cells that secrete insulin will be required. In this respect, the most recent (though still very early) advances in stem cell research are encouraging: islets or islet cells have been produced from adult human pancreatic ductal stem cells [70], as well as from embryonal human [69] and mouse [67, 68] embryos. What then are the possibilities for successful gene treatment of type 1 diabetes? We are cautiously hopeful. However, adherence to some fundamental engagement guidelines and a better comprehension of some significant events will be necessary for success. Let's hope that the necessary sectors will see rapid advancement and discovery.

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