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Review Article

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Examining Potential Type I Diabetes Mellitus Treatment Options – A Molecular Approach

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Abstract

The last few decades has seen monumental strides in both technologic and scientific advances and discoveries in the field of diabetic research. This review article discusses the background behind Type I Diabetes Mellitus (T1DM), how it is an autoimmune condition with a molecular origin dysfunction before presenting discussion on recently discovered concepts. The article explores the role that stem cells play in diabetic treatment beginning with graft harvesting before discussion of newly discovered stem cells in the spleen and what that means for treatment. Tumor necrosis factor alpha (TNF- α) is believed to play a role in therapeutic options for diabetics, as there is reason to believe that TNF-α is capable of inducing apoptosis in selectively autoreactive CD8$^+$ T-cells and data behind utilizing TNF agonists is illustrated. Ultrasensitive c-peptide assays shed light on the true functional status of islet β cells and conclude that the decline in function occurs over decades and not months as was previously thought. All these concepts and discoveries pave the way for future clinical trials and the discovery of more curative diabetic treatment options.

Keywords: Type I Diabetes, Stem Cells, TNF-α , C-peptide assays, Endocrinology, Metabolism

Introduction

Type I Diabetes Mellitus (T1DM) is an autoimmune disease that affects the pancreas and normal levels of insulin secretion. Insulin, an endogenous hormone that is produced by the pancreas, is essential for normal physiological functioning of the body and plays a vital role in the regulation of the body's blood glucose levels. In a patient with T1DM, the immune system goes rogue and attacks the insulin-producing islet cells of the pancreas specifically called β-cells. T1DM is the more aggressive version of the disease as compared to Type II Diabetes Mellitus (T2DM) and begins most commonly in childhood.

Human autoimmune diseases are very difficult to treat because of their inherent mechanism of attack. The body's own cells sabotage themselves and turn hostile within their own system. A common treatment option for treatment of autoimmune conditions is the utilization of immunosuppressive drugs. These drugs act on the patient's immune system and, ideally, cut off the resources that rogue immune cells thrive on in order to establish another drug or treatment option for their eradication. This meant that these class of drugs had to conform to a wide spectrum of cell structures. As a result of this, immunosuppressive drugs are very nonspecific because of the fact that they need to encompass a broad range of cells. Immunosuppressive drugs also produce very high levels of adverse effects because of their uniform conformity to several other cells and the effects that these interactions proliferate. The crucial obstacle that immunosuppressive drugs could not surmount was the fact that they were not an effective therapy for the destruction of the body's hostile autoimmune cells. However, this approach was an important stepping-stone upon which other novel technologies and researchers could springboard off to provide much more ameliorative care.

Within the last decade, there have been monumental strides in both the fields of technology but also in the field of molecular genetics and biology. This, in turn, has led to copious novel ideas that were previously thought to be unfeasible in providing viable treatment options for T1DM. The objective of this paper is to provide a synthesis and highlight a couple very unique and innovative therapies that have potential to be used as future cure plans for patients suffering from T1DM. Specifically, the focal point of the research, which will be summarized and delineated within this paper, will underscore the molecular aspect of treatment options for several reasons. The first being that the abnormality of T1DM is of molecular origin; thus, formulating a molecular therapy would be the most logical and effective strategy. When molecular treatment options are created they not only act to address a preventive side that effectively acts to significantly reduce the likelihood of contracting T1DM, but also molecular therapies are efficient as potential strategies for the reversal of the progression of the disease. The combination of both of these benefits provide very promising results and aim for a multi-pronged approach for both prevention and reversion of T1DM.

Review of Literature

Islet Cells

Autoimmune destruction in pancreatic β cells is more than 90%

complete by the time hyperglycemia becomes clinically evident in individuals with T1DM. Prevention of this disease would therefore optimally require arrest of autoimmunity in the pre-hyperglycemic phase. By targeting symptomatic patients in the pre-hyperglycemic phase, this will selectively increase the rate of prevention due to the fact that there are higher functioning cells still intact in the body [10]. Figure 1 below shows the molecular result of T1DM when there are various other ailments within the body – such as a metabolic deficiency or a defective immune tolerance.

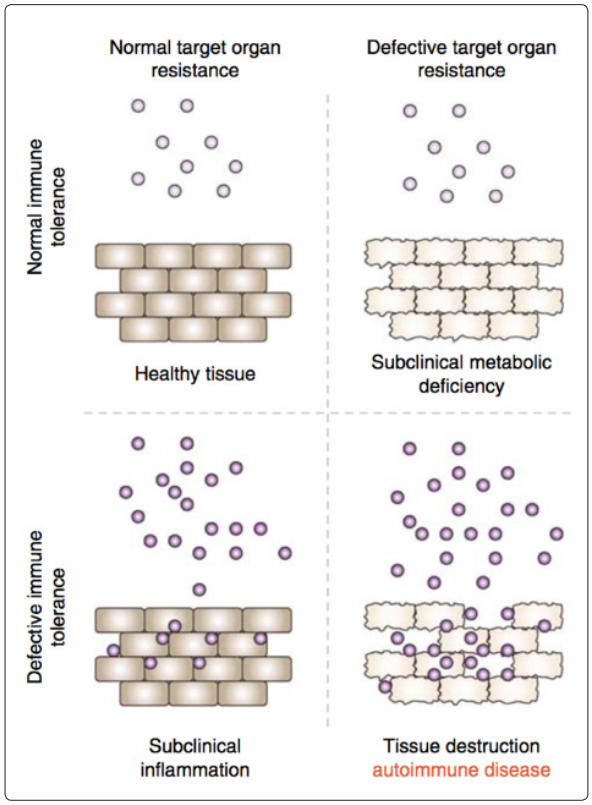

Figure 1: Healthy tissue is dependent on normal immune tolerance and target organ resistance [10]

However, there needs to be insult to both of these aspects in order to produce clinically significant outcomes. This synergism owes to the fact that a breakdown in immune tolerance or target organ resistance yields either subclinical tissue inflammation or subclinical metabolic deficiency, respectively. It is only when both these aspects fail does the autoimmune insult and subsequent tissue destruction ensue.

A popular treatment plan is to implant a graft of exogenous islet cells into the patient with the hope that the extra boost of islet cells will contribute to the normalization of blood glucose levels. The immune mechanism of islet graft rejection and recurrent autoimmunity appear distinct, and protective interventions targeted at these two pathways of islet destruction are non-overlapping in effectiveness. Researchers examined the hypothesis that induction of tumor necrosis factor alpha (TNF- α) expression combined with re-education, a process by which antigen presenting cells display biomarkers that results in the death of potentially pathogenic cells, of newly emerging T-cells with self-antigens can interrupt autoimmunity. TNF-α was merely pre-empted in this study and will be further analyzed in subsequent therapies. To bypass graft rejection, donor islets were used from C57BL/6 mice in which the β_2 M gene was deleted and the major histocompatibility complex (MHC-I) proteins are re-expressed on graft cells. With regard to interruption of autoimmunity, Complete Freund's Adjuvant (CFA) treatment might eliminate the auto-reactive lymphoid cells of non-obese diabetic (NOD) mice by promoting their apoptosis, in part through the induction of TNF- α. Current data from studies conclude by saying that these interventions reversed the established β cell-directed autoimmunity and restored endogenous pancreatic islet functions to such an extent that normoglycemia was maintained in up to 75% of animals after discontinuation of treatment and removal of islet transplants. This finding corroborated the claim that certain steps could be taken for the reversion of T1DM.

Further research discusses possible biological therapies for T1DM treatment highlighting 4 mechanisms that could be potential uses for regeneration, but places strong emphasis on 2 – β-islet proliferation and stem cell differentiation [2]. The research demonstrates how stem cells are a much safer way of potential treatment options for T1DM due to their lower risk of immune rejection. Researchers screen for certain cells that meet 2 essential criteria to them: self-renewal properties and the capacity to give rise to a range of specialized cell types. Thus, for this reason, stem cells seem to be the most appropriate candidate. The researchers then spend a lot of time delineating a serendipitous finding that their research lab came across - a very large stem cell population in the spleen. Further experiments proved that splenocytes taken from donor mice could contribute to the reversal of the autoimmune defect in NOD mice. They also showed that donor spleen cells resulted in very rapid islet regeneration. Not all spleen cells were capable of forming new islets in diabetic mice; only non-lymphoid spleen cells (CD45- cells) could directly contribute to the newly formed islet cells [3]. CD^{4+} T-cells are a subset of T-cells not directly responsible for killing target cells, but rather recruit other immune cells responsible for battle. Extensive experimentation revealed that the host's islets could regenerate spontaneously, although more slowly, without any cellular therapy, as long as the underlying autoimmune disease was eliminated – spontaneous regeneration was sufficient by itself to allow for islet regeneration.

Expanding on the findings from previous research, this research states how NOD mice exhibit spontaneous autoimmunity that cause diabetes through destruction of insulin-secreting pancreatic islets [4]. A lymphoid cell-specific proteasome defect in these mice interrupts the presentation of self-antigens by MHC-I proteins that are required for negative selection of auto-reactive naïve T-cells. This inactivation of negative selection is the reason why the body doesn't target defective pathogenic cells for either destruction or apoptosis. This concept is graphically illustrated in figure 2 below [5]. The researchers injected NOD mice with splenocytes to see what effect they would have *in vivo*. Similar to previous data, 6/9 (67%) NOD mice that received live splenocytes remained normoglycemic after removal of the islet transplant. Conversely, none of the eight mice that received irradiated splenocytes remained normoglycemic; they all rapidly developed severe hyperglycemia. Of the 12 NOD mice that received live splenocytes, 11 (92%) remained normoglycemic for >26wks after disease onset or beyond 52wks of age in duplicate trials. In conclusion, the research states that therapies to reverse autoimmune diabetes need not incorporate transplantation of exogenous adult islets.

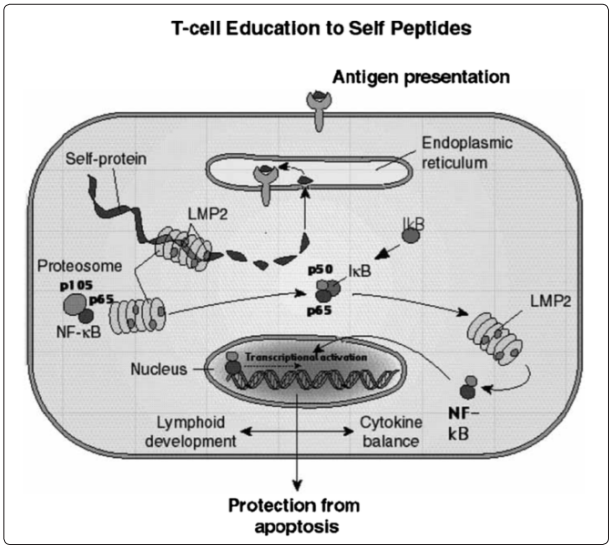

Figure 2: The figure above depicts the process of antigen presentation by MHC-I on an antigen-presenting cell highlighting the specific role the proteasome, LMP2 plays in two biochemical pathways that may be relevant to T1DM. Cytoplasmic proteins that are degraded by the LMP2 proteasome are then transported to the rough endoplasmic reticulum where they are loaded onto the MHC-I protein and subsequently delivered to the cell surface via vesicles from the Golgi apparatus. LMP2 also plays a role in activating the nuclear transcription factor KF- *k*B, which is crucial for lymphocyte maturation and regulating cytokines that play a role in apoptosis [5].

These findings help to significantly elucidate the understanding of T1DM. Prior to these experiments, it was always understood that there needed to be a 2-pronged approach for potential T1DM treatment options. The first being the eradication of the cells responsible for the underlying autoimmune attack on the body and the second to find ways to catalyze the regeneration of the host's islet cells. However, the conclusion of the research debunks the later claim and illustrates that the body is fully capable of self-recovering the islet cells [5]. The former problem still applies, as regeneration is only feasible when the underlying autoimmune attacks have stopped. The researchers also discovered a large pool of splenic stem cells that were previously undiscovered and might have a substantial effect on treating the autoimmune aspect of T1DM.

Looking to the Spleen

It has been long established that the spleen contains a reserve population of hematopoietic stem cells that are tapped when the bone marrow cannot fully meet the body's demands [6]. A hematopoietic stem cell is a very specific type of stem cell that is not restrictive in its lineage. In other words, a hematopoietic stem cell is a stem cell that has the capability to give rise to all the other blood cells. An experimental study by Krapp and co-authors found that knockout mice lacking a pancreas (due to the ablation of PTF1-p48) were born normoglycemic; their endocrine-derived β -islet cells had developed in the spleen instead of their usual location the pancreas, which was missing. This finding is also commonly seen and can be extrapolated into the field of medicine, as insulin - dependent diabetes is the eventual outcome for individuals with pancreatitis who had a left-hemipancreatectomy, but not a right-hemipancreatectomy. Left-hemipancreatectomy requires removal of the spleen along with the pancreas owing to their shared vasculature, whereas a right-hemipancreatectomy does not involve spleen removal. Similarly, spleen removal in children with severe thalassemia's leads to the eventual development of insulin-dependent diabetes. Thus, a clear physiological link can be observed between the pancreas and spleen.

The spleen is an organ that harbors populations of newly identified stem cells that can differentiate into fully functional insulin-producing islet cells of the pancreas [7]. The importance of the spleen is further reinforced by the finding that one population of splenic stem cells express a key embryonic transcription factor, Hox11, which regulates organogenesis and participates in the development of the nervous system. Experiments revealed that splenic cells, after being harvested from donor mice and co-injected into a diabetic host with a drug that induces TNF (for the selective killing of pathogenic T-cells), migrated to the pancreas and differentiated into fully functional cells that normalized blood glucose levels. Human pancreatic stem cells take up long-term residence in the mouse spleen, in preference to mouse bone marrow and peripheral blood. The human cells remain in the mouse's spleen for at least 60 days, which is a period far longer than would be expected if the spleen were merely the site of passive blood filtration. The connection between the pancreas and spleen goes even further as decades of descriptive embryology reveals that the spleen and pancreas are formed at the same time in close proximity – sharing a mesodermal lineage [7]. The spleen anlage (embryonic tissue destined to become spleen) relocates during gestation to the mesenchyme of the pancreas, without colonization by hematopoietic cells. Hox11 stem cells offer a unique gateway into potential candidates for treating T1DM because of the fact that they share commonality between the pancreas and the spleen. This broadens the scope of the search to include organs that have these commonalities that the spleen and pancreas share and is the reason why researchers took an abrupt interest in evaluating the spleen. The discovery that the spleen shares the transcription factor Hox11 only added to the credibility of using the spleen for potential therapy options.

Hox11+ stem cells hold potentially broader therapeutic applications because they are less lineage restricted due to the fact that they do not express the hematopoietic marker CD45+. Hox11+ stem cells from the spleen of normal mice have been harvested to assist in both treatment and cure of T1DM and Sjogren's syndrome, another autoimmune disease [8]. Hox11 contains a proliferative role and is responsible for the development of wide-ranging organs and tissue structures. The spleen is also the only bodily organ that houses a reservoir of Hox11 expressing stem cells. One caveat when dealing with Hox11 is the possible risk of stem cell transformation into cancer genes. This is especially apt with Hox11 because it was identified as a proto-oncogene in human T-cell acute lymphoblastic leukemia. Consequently, potential donors of Hox11 stem cell therapies could be analyzed and genotyped for chromosomal translocations to reduce the possibility of tumorigenesis. The main conclusion is that removal of the underlying autoimmune disease is essential to see the regeneration of the pancreas, salivary gland, or even portions of the inner ear, as in the case of Sjogren's syndrome.

Expanding onto previous research, researchers discovered that an immune therapy triggered a permanent reversal of end-stage T1DM in mice [9]. This treatment took a 2 pronged approach for maximum efficacy and feasibility. The first component was injecting mice with an immune adjuvant, such as CFA. This induced the production of

TNF, which aids in the destruction of auto-reactive T-cells, which are defective subset of T lymphocyte cells that attack and kill the body's own regular T-cell targets such as insulin. The second component was injecting splenocytes from a donor mouse. Researchers noticed the regeneration of pancreatic islets and the complete reversal of T1DM without the introduction of any live donor splenocytes. These findings are corroborated by other studies that show that the regenerative process in the pancreas is likely to be intact and that targeted immune intervention may unleash the spontaneous regeneration of the pancreas. Multiple research studies concluded that the regeneration of the pancreas once the autoimmune process was removed possible. Thus, it is necessary to examine how TNF and its pathways affect normal and pathogenic T-cell response in order to devise treatment options for T1DM that selectively kill auto-reactive T-cells.

TNF and its Connection to Activate Selective Apoptosis Pathways

Several recent studies have suggested that for the pancreatic β islets, the target organ may be defective and that these defects may be involved in the development of diabetes [10]. Further studies revealed abnormal development of pancreatic tissue, increased insulin production, and profound insulin resistance. NOD pancreatic islets also show an intrinsic resistance to $CD8^+$, T-cells that are directly responsible for killing their targets, mediated destruction, a trait that is genetically linked to TNFR2, a specific variant of TNF, within the diabetogenic Idd9 genetic loci. In addition to the defects observed in the pancreas, studies observe other structural defects in the cochlea, salivary glands, and the tongue of NOD mice, which appear to be primary defects of autoimmune conditions rather than secondary changes due to autoimmunity. What is also interesting to note is the fact that the above symptoms are all dependent on Hox11 for development – underscoring the finding that the observed defects in NOD mice have a common genetic causality.

TNF is a key signaling protein in the immune system. As a regulatory cytokine, TNF orchestrates communications between immune cells and controls many of their functions [11]. TNF is best known for its role in leading immune defenses to protect localized area from invasion or injury but it is also involved in controlling whether target cells live or die. TNFR2 signaling involves the mobilization and nuclear entry of the transcription factor NF- *k*B to promote transcription of pro-survival genes, which T-cells exclusively rely on. Auto-reactive T-cells have been found to be sensitive to death, induced by exogenous, low-dose TNF exposure. This is due to a defect in the activation of NF- B, which means that pro-survival genes are not transcribed in response to TNF and apoptosis of these cells is instead favored, as can be seen in figure 3 below. In one cell type, TNF administration *in vitro* sequentially induces pro-apoptotic then pro-survival effects. TNFR1 activates the caspase family, which induces cell death. After TNFR2 binding, the nature of the functional effects can depend on which of the genes NF- *k*B transcribes. As TNFR1 and TNFR2 are co-expressed on the same cells, evidence points to a certain degree of crosstalk between the receptors.

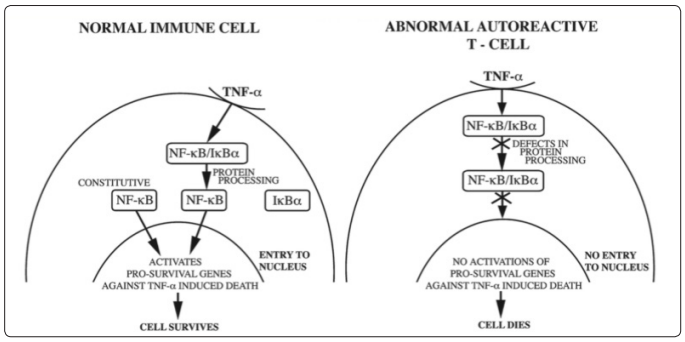

Figure 3: TNF- exposure response in normal and autoreactive T-cells. In normal T-cells NF- B is complexed with I B- preventing nuclear sequestration of NF- *k*B. Once TNF- α binds to the T-cell, proteasomes break this NF- *k*B and I*k* B-α complex allowing for nuclear translocation to happen and the transcription and activation of pro-survival genes to counteract the apoptotic effects of TNF- α. However, in autoreactive T-cells, proteasomal defects prevent the dissolution of the NF- *k*B and I*k* B-α complex not letting nuclear sequestration of NF- *k*B. Preventing this nuclear sequestration means the cell cannot activate pro-survival genes and ultimately leads to the death of the cell [7].

Apoptosis could play a role in autoimmune disease under two different guises first, controlled regulation of cell death is a normal part of T-cell selection and T-cell education [12]. Interruption of this process could lead to auto-reactive cells. Second, cell death could represent a lymphocyte-independent mechanism causing cells in certain organs to die prematurely. Most recent data has shown that a defect in the proteasome subunit's, LMP2, protein expression not only prevents the effective generation of self-peptides for MHC class I protein display, but also interrupts the proteolytic processing of NF-*k*B, a protein crucial for T-cell maturation and for normal immune and inflammatory response, as well as degradation of the inhibitory I *k*B subunit. This proteasome defect reduces the generation of active NF- *k*B, which regulates lymphocytic maturation, normal regulation of T-cell pro-inflammatory and cytokine production and protection of T-cells from TNF-α induced apoptosis. The defective NF- *k*B activation is confined only to those lymphoid lineage cells that lack effective LMP2 protein expression. These studies established for the first time that MHC linked genes such as LMP2 can have a direct role in T-cell development and apoptosis. The findings demonstrated the existence of a marked defect in proteasome function in lymphocytes from autoimmune diabetes-prone NOD mice. This dysfunction results from a lack of the LMP2 subunit, which is encoded by a gene located in MHC region of the genome, and it results in both impaired processing of self-peptides for presentation by MHC class I molecules as well as the inability to activate NF- *k*B.

Excess levels of TNF-α have been associated with certain autoimmune diseases. Contrary to logical thought, boosting or restoring TNF-α activity- rather than blocking it- might be therapeutic for some forms of autoimmunity because of its ability to selectively kill, by apoptosis, auto-reactive (pathogenic) T-cells but not normal cells. This is due to the fact that auto-reactive T-cells continue to remain sensitive to TNF- α induced apoptosis; thus, treatment with TNF- α appears to be a highly targeted strategy to destroy auto-reactive T-cells and interrupt the pathogenic autoimmunity [13]. Studies suggest that several autoimmune diseases have a

common vulnerability to TNF-α exposure: their activated, auto-reactive T-cells are sensitive to TNF-α induced apoptosis. The common vulnerability appears to be linked to various erros in NF-*k* B signaling. The possible therapeutic benefit of TNF-α therapy is well demonstrated in NOD mice models. With either direct TNF-α administration or TNF- α-induction, murine autoimmune disease is reversed. Conversely, in the same mouse model, blockade of TNF signaling accelerates autoimmune disease.

Using human blood specimens, research experiments tested the addition of TNF, or more selective agonists of TNF, for their capacity to kill only auto-reactive T-cells in several autoimmune diseases, while sparing normal T-cells. In isolated $CD4^+$ T-cells, no TNF inducing killing was observed in either diabetic or control samples. TNF induced mild cell proliferation but not cell death. Conversely, TNF killed $CD8^+$ T-cells but not control $CD8^+$ T-cells at all TNF doses. This finding suggests that TNF induced killing of a subpopulation of $CD8^+$ T-cells extends to other autoimmune diseases. TNF acts by binding to one of two cell surface markers, TNFR1 or TNFR2, although the intracellular machinery associated with these receptors is dissimilar. Even though the apparent function and mechanism for TNFR1 and TNFR2 might be different, studies have shown that there is a way for the TNFR1 pathway to cross over into the TNFR2 and vice versa, as can be seen in figure 4 below. TNFR1 is ubiquitously expressed on all T-cell populations, the entire lymphoid system, and most other cells. This explains TNF's systemic toxicity when used at high doses in oncology treatments. TNFR2, in contrast, is more restrictively expressed, found only on select subpopulations of T-cells, endothelial cells, neurons, and other occasional cells. These research studies showed that TNF exposure kills a subset of human $CD8^+$ T-cells from T1DM and other autoimmune diseases [12]. Conversely, $CD4^+$ T-cells from T1DM are resistant to TNF triggered death. Death of $CD8^+$ T-cells is triggered with a specific agonist for TNFR2 that mimics TNF's actions, as can be seen in figure 4 below. TNFR1 agonists do not trigger cell death of auto-reactive T-cells. However, a subpopulation of $CD8^+$ T-cells specific for the HLA class I insulin fragment did die upon exposure to the TNFR2 agonist.

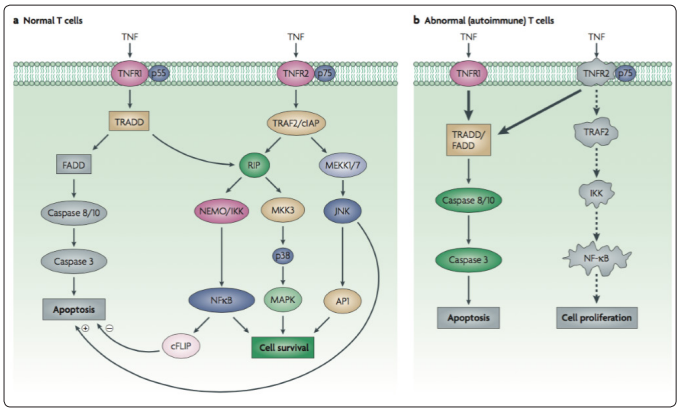

Figure 4: TNF signaling in normal and autoreactive T-cells. (A) TNF can exert different effects on the same cell depending on which TNF receptor (TNFR) it is bound to. Exclusive binding to TNFR1 triggers a downstream signaling cascade that results in the cell undergoing apoptosis. Conversely, exclusive binding to TNFR2 stimulates a different downstream signaling cascade that facilitates cell survival pathways that produce cellular proliferation. (B) In autoreactive T-cells there are more defects within the TNFR2 pathway proteins that makes binding to TNFR2 less efficacious than normal. These defective TNFR2 proteins bias autoreactive T-cells to preferentially bind to TNFR1 facilitating apoptosis of the cell [3].

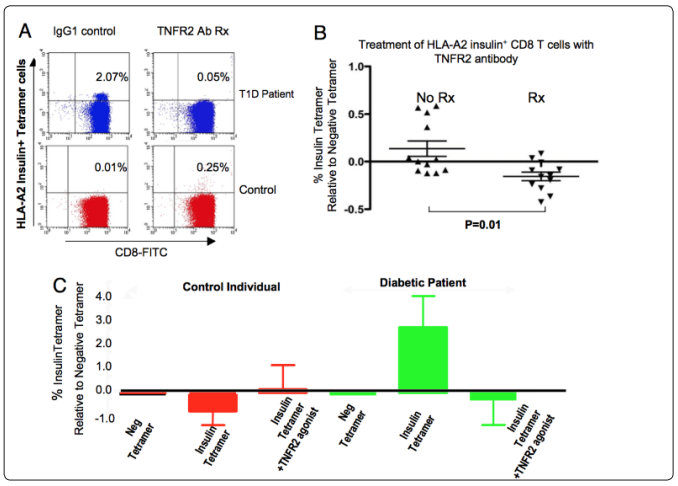

Figure 5: Treatment of insulin autoreactive $CD8^+$ T-cells with TNFR2 agonist eliminates autoimmune cells. (A) Elimination of insulin B10-18 tetramer T-cells with a 6-hour treatment with TNFR2 agonist in a long-term diabetic compared with controls. (B) Insulin B10-18 specific $CD8^+$ T-cells from T1DM decreased in culture with TNFR2 agonist treatment in culture for 6 hours. (C) Repeated TNFR2 agonist treatment over a 3-year treatment consistently shows elimination of autoreactive $CD8^+$ T-cells [12].

So far, this paper has focused on research that deals with possible methods of eradication of auto-reactive $CD4^+$ and $CD8^+$ T-cells. However, it would be remiss to neglect to summarize research done on C-peptide production levels in the pancreas because even if the underlying autoimmune aspect of the disease was removed, there would not only still need to be some islet cells remaining, but also they would need to be functional in order to maintain normal glycemic control.

C-Peptide Production Levels and their Clinical Significance

Improved biomarkers that predict patient vulnerability to diabetic complications might enable targeted therapeutic strategies to be implemented. Recent evidence shows that pancreatic β cells are frequently still viable and functional decades after onset of T1DM based on new ultrasensitive C-peptide assays. C-peptide is secreted at a 1:1 molar ratio to insulin, thus representing a direct measure of endogenous insulin. An independent association was found to exist between low levels of C-peptide assays and complications. More specifically, a C-peptide level >10 pmol/L was protective from complications, and a value of C-peptide <10 pmol/L presented a risk of complications. Consequently, low C-peptide levels (2.5-5.0 pmol/L) were associated with higher HbA1C values (median 55mmol/mol; 7.2%) compared with substantially more C-peptide at levels of 50-100 pmol/L (median 52mmol/mol; 6.9%). Low levels of residual fasting C-peptide may have clinical significance in preventing complications and impact on HbA1C. In an analysis of 324 patients, preservation of C-peptide levels >10 pmol/L was associated with protection from the onset of diabetes-specific complications. Islet transplant studies have also shown that low levels of endogenous stimulated C-peptide (>30 pmol; L) may assist in maintaining fasting blood glucose values, lower HbA_{1C}, and prevent severe hypoglycemia. Thus, detection of C-peptide levels serves not only as a preventive measure for people just entering the

pre-diabetic phase, but also can detect the likelihood of possible complications that might arise for patients already affected by T1DM.

Researchers were able to find that β cells remain functional even in subjects with low C-peptide output [14]. As disease duration increased, C-peptide levels tended to gradually decline, but this was a decline over decades of disease – not a decline over months, as is commonly viewed to be the course of the pancreas. Other findings support past population-based studies that any preservation of C-peptide is associated with decreased complications. The data show that C-peptide levels can be present for decades after disease onset, suggesting that long-term preservation of pancreatic function and C-peptide production is not confined to a short time (~1yr) after clinical onset but, rather, persists in some cases beyond 30 years. Also, the study found that sex was unrelated to C-peptide levels. In summary, the results revealed that 1) the ultrasensitive assay found that 10% of patients with disease duration of 3-4 decades still produced C-peptide; 2) β cell functioning was intact at C-peptide levels as low as 2.8 ± 1.1pmol/L; 3) longer disease duration was associated with lower levels of C-peptide; and 4) adult onset diabetes with onset at >40 years of age showed a more rapid loss of C-peptide levels despite relatively short disease duration. These results are very promising as they allow more research to be conducted into the difficult task of initially eradicating the underlying autoimmune aspect of conditions like Sjogren's syndrome and T1DM [14].

Human Trials

This synthesis section is the scarcest out of all of the others due to the fact that there is only one research lab that made it to a phase II human trial scenario that deals with treating T1DM on a molecular level that addresses both prevention for people unaffected by the disease and complete reversion for those who are. Such trials are very rare but hopefully will become more frequent over the course of the few years that will follow. Technology has grown to such a revolutionary point that we now know the things that no one would have predicted to be known 50 years ago. It is with this final research summation that the paper will leave the reader with, as it not only encompasses and utilizes all of the research concepts discussed above, but also serves, as a beacon of hope for the promising therapies to come that will treat this malady of the century [15].

In the case of T1DM, the rationale for administering TNF is that insulin-auto-reactive T-cells bear several intracellular signaling defects that make them selectively vulnerable to death upon exposure to TNF. However, TNF treatment at high doses in humans is limited by its systemic toxicity. Thus, researchers used a FDA approved vaccine containing *Mycobacterium bovis bacillus – Calmette – Guerin* (BCG), which is a very common and well-known tuberculosis vaccine [15]. A single, low dose of BCG in humans with late-stage pre-diabetes was initially found to successfully induce a clinical remission in some patients but it could not be observed a year after vaccination. Cytometry study of dead and living insulin auto-reactive T-cells revealed that pathogenic T-cells captured in the blood had both the common low affinity insulin auto-reactive cells as well as the treatment-specific release of high affinity auto-reactive T-cells for the insulin peptide fragments. Thus, by week 5, only a week after the BCG injection, dead insulin auto-reactive T-cells appear in the patient's blood. Two of the three BCG-treated subjects had transient increases in fasting C-peptide levels by week 20 compared to either their baseline or to the values in the reference diabetic subjects. BCG ameliorates the advanced autoimmune process underlying T1DM by stimulating TNF, which selectively kills only disease-causing cells and, further, permits pancreas regeneration as evidenced by the transient increase in C-peptide secretion observed using an ultrasensitive C-peptide assay. More investigation needs to be performed as to the dosage amounts; suggesting that a larger dose of BCG might be more effective. The transient increase in C-peptide suggests a positive impact of these immune perturbations on beta cell function.

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