

# Prevention of Type I Diabetes Mellitus: The Role of Immune Interventions

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## Abstract

Diabetes mellitus is a disease of antiquity with an estimated 20,000 people dying prematurely per year due to diabetes associated disease. Type 1 diabetes results from autoimmune destruction of insulin-producing pancreatic cells. Much effort has been dedicated to figure out the autoimmune background of this disease with a view to exploring how the immune system can be manipulated to prevent its occurrence. In this review, we explore the autoimmune basis of type I diabetes mellitus and equally the immune interventions that have been and are being employed to prevent type I diabetes mellitus.

## Introduction

Diabetes mellitus is a chronic endocrinological disorder manifesting as high blood glucose levels either due to insufficient secretion of insulin by the pancreas or improper utilization of insulin by target cells [1]. Diabetes mellitus affects over 230 million people worldwide with an estimated global prevalence of 5.1% [2]. Two types of diabetes mellitus exist viz; type I or insulin dependent diabetes mellitus (IDDM) and type II or non-insulin dependent diabetes mellitus (NIDDM) [3].

The incidence of type 1 diabetes in children younger than 15 years is increasing. If present trends continue, doubling of new cases of type 1 diabetes in European children younger than 5 years is predicted between 2005 and 2020, and prevalent cases younger than 15 years will rise by 70% [4]. Although type I and type II diabetes differ greatly in modes of pathogenesis, these disorders share a common pathology and consequences characterized by loss of functional  $\beta$ -cell mass and subsequent dysregulation of carbohydrate and lipid metabolism [2].

The majority of the patients are diagnosed and classified with type I diabetes within the first two decades of life, but an increasing number of cases are being recognized in older individuals [5]. Even though the precise cause of the disease remains unclear, a combination of genetic, immunologic, and nongenetic factors contributes to the onset and progression of IDDM [6,7]. The major form of type I diabetes (T1D) is characterised by immune-mediated pancreatic islet  $\beta$ -cell destruction, and has also been called type IA diabetes to distinguish it from idiopathic forms of islet  $\beta$ -cell loss [8]. Therapy for diabetes and the associated complications poses enormous public health and economic burdens [2].

Despite recent progress in therapy and management of diabetes mellitus, diabetes remains a serious disease with life-threatening complications. It is by far the most common metabolic disease [9].

In type I diabetes mellitus, insufficient production of insulin is caused by the chronic autoimmune destruction of the insulin-producing  $\beta$ -cells [Orban and Kis, 2011]. The initial immune response engenders secondary and tertiary responses which, together, end in impairment of  $\beta$ -cell function and progressive destruction of  $\beta$ -cells thereby resulting in the development of type I diabetes mellitus [8]. It is an insidious process that may occur over years. During the stage of disease evolution (prediabetes), individuals may be identified by the presence of immunological markers and a decline in beta-cell function [9].

The autoimmune nature of the disease process has invoked efforts aimed at arresting the disease process by immune intervention strategies. Over the last quarter century much investigation has been directed at interdicting the type I diabetes disease process, both during the stage of evolution of the disease and at the time of disease onset [8,9]. An avalanche of screening programs is used to identify high-risk subjects who may benefit from an early intervention, with the ultimate goal of curtailing the development of overt type I diabetes mellitus in those at risk for the disease, using both specific and safe strategies. These emerging novel preventive and regenerative therapies are aimed at preserving  $\beta$ -cell mass and delay the onset of diabetes. [2,9]. Thus, there have been many studies designed to interdict the T1D disease process, mostly by altering the immune system, both during the stage of evolution of the disease and at the time of disease onset [8].

## The Role of Autoantibodies in Type I Diabetes Development

The presence of pancreatic islet cell autoantibodies confirms that type I diabetes is autoimmune in origin. The disease process is primarily caused by the destruction of pancreatic beta cells. This cell destruction is thought to result mainly from the action of T-lymphocytes, the key players in autoimmune disease development. The beta cell autoantibodies that characterize type I diabetes may not be responsible for cell destruction. Instead, these antibodies are thought to signal a T-cell mediated immune response that sets the stage for beta cell destruction [10].

Islet cell antibodies were the first autoantibodies discovered in patients with diabetes. However, antibodies specific to the beta cell antigens that make up islet cells are more specific. Antibodies to insulin and proinsulin also occur in diabetes. Antibodies to the enzyme

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glutamic acid decarboxylase (GAD), which is found in nervous system and pancreatic cells, are also present in diabetes. GAD antibodies were first demonstrated in patients with Stiff-Man syndrome, a disorder sometimes observed in patients with diabetes. Antibodies to the islet cell protein tyrosine phosphatase (IA-2) and phogrin, are also seen in diabetes [10,11].

Recently, the human zinc transporter Slc30A8 (ZnT8) has been developed as one of the major target of humoral autoimmunity in human type 1A diabetes. However, despite extensive conservation, the majority of human autoimmune sera fail to recognize the murine ortholog. Moreover, Slc30A8 appears not to be a significant target of humoral autoimmunity in the NOD mouse. Therefore, the murine protein was "humanized" by site-directed mutagenesis [12]. Only conversion of Q324 to arginine (equivalent to R325 in the human protein) partially restored reactivity to a pool of sera selected for high titers to the human probe. Additionally, the reciprocal mutation (human R325 to Q) abolished reactivity for 38/103 (36.9%) of ZnT8+ sera. It was concluded that the C-terminal domain of human ZnT8 contains at least two discrete epitopes, one of which is critically dependent upon the arginine residue at position 325 [12].

### Autoimmune Background of Type I Diabetes Mellitus

Since the first demonstration of islet cell antibodies in 1974, the concept has been that this form of diabetes has an autoimmune background. The commonly accepted concept is that antibodies, representing the humoral immunity, do not mediate the  $\beta$ -cell destruction but instead serve as markers of that destruction, while the cellular arm of the immune system, specifically T-lymphocytes, mediate the  $\beta$ -cell destruction. However, the T-lymphocytes hardly act alone. They are aided in initiating the response by antigen-presenting cells such as dendritic cells and macrophages. Apparently, they are also helped by B-lymphocytes [8].

In addition, the initial immune response causes secondary and tertiary responses – involving the whole immunological army - which collectively result in impairment of  $\beta$ -cell function, progressive destruction of  $\beta$ -cells, and consequent development of type 1A diabetes. The process is menacing and may evolve over several years, with the overt expression of clinical symptoms becoming apparent only when most  $\beta$ -cells have been destroyed [5].

And, although it has been thought that ultimately there is complete  $\beta$ -cell destruction, several studies have now demonstrated some degree of persistent  $\beta$ -cell function or existence (at autopsy) in long-standing T1D. A major focus of investigation in T1D is the preservation of  $\beta$ -cell function (and, it is hoped, of  $\beta$ -cells themselves), in the expectation that continuing endogenous insulin secretion will contribute towards better glycaemic control, reduce episodes of severe hypoglycaemia, and slow the development of complications such as retinopathy and nephropathy [5].

Autoreactive T cells, both CD4 and CD8 cells, have been implicated as active players in  $\beta$ -cell destruction. A series of autoantigens have been identified in type 1 diabetes including insulin, GAD, IA-2, and most recently the zinc transporter Slc30A8 residing in the insulin secretory granule of the beta-cell [13]. In spite of the critical role of antibodies for the diagnosis of the disease in patients, most data suggest that T cells are the key players in the autoimmune attack of  $\beta$ -cells [14]. Anti-islet T cells, both CD4 and CD8 T cells, have been identified in type 1 diabetic patients as well as in the animal models [15].

Importantly, transfer of anti-islet specific CD4 or CD8 T cells induces diabetes to immuno-incompetent recipient Non-Obese Diabetic (NOD) mice. In contrast, antibodies do not transfer the disease. CD8 T cells can directly kill  $\beta$ -cells that express Major Histocompatibility Complex (MHC) class I, through perforin/granzyme secretion. CD4 T cells that recognize peptides presented by MHC class II molecules usually participate in carrying out  $\beta$ -cell destruction directly by the production of interferon- $\gamma$  (IFN- $\gamma$ ) and indirectly by the activation of local innate cells such as macrophages and dendritic cells [16]. Conversely to effector T cells, another T-cell population dampens autoimmune pathological responses. Regulatory CD4 T cells, expressing the molecule forkhead box P3 (Foxp3), inhibit the development of diabetes [17].

The protective role of this population has been clearly demonstrated in the NOD mouse. Patients harboring mutations in the *Foxp3* gene can develop several autoimmune diseases including T1D [18]. These observations confirm the role of this regulatory T cell population in humans. Even though B cells are not required for the effector phase of T1D, several studies have revealed the role of these cells in the development of the disease. B cell deficiency by gene targeting and B cell depletion by specific antibodies prevent the development of the disease in NOD mice [19]. Similar treatment improves the  $\beta$ -cell function in newly diagnosed patients [20].

### The Role of Innate Immune Cells in Type I Diabetes Mellitus

Two arms of the immune system, innate and adaptive immunity, differ in their mode of immune recognition. The innate immune system recognizes a few highly conserved structures on a broad range of microorganisms. On the other hand, recognition of self or autoreactivity is generally confined to the adaptive immune response. Whilst autoimmune features are relatively common, they should be distinguished from autoimmune disease that is infrequent [21]. Type 1 diabetes is an immune-mediated disease due to the destruction of insulin secreting cells mediated by aggressive immune responses, including activation of the adaptive immune system following genetic and environmental interaction. Hypotheses for the cause of the immune dysfunction leading to type 1 diabetes include self-reactive T-cell clones that escape deletion in the thymus, escape from peripheral tolerance or escape from homeostatic control with an alteration in the immune balance leading to autoimmunity [21].

To avoid harmful chronic inflammatory and autoimmune responses to the host, the immune system requires precise regulation when mounting effective immune responses to fight infections while maintaining homeostasis and tolerance to self-components [22,23]. The innate immune system employs macrophages, dendritic cells (DCs), natural killer (NK) cells, natural killer T cells (NK T) and  $\gamma\delta$  T cells, which recognize potentially dangerous molecules via germ line encoded receptors and elicit rapid immune responses that function as the first line of defense against foreign substances and also lead to the stimulation of antigen-specific cells of the adaptive immune system [24].

Among them, NK T and  $\gamma\delta$  T cells may reside between the innate and adaptive immune systems because they also bear receptors encoded by somatically rearranged genes like adaptive immune cells, while they generally lack the potential for establishing antigen-specific clonal memory cells. The type of immune response mediated by such

cells has been referred to as transitional immunity. Components of the innate immune system have been established to play a major role in the development of sustained and adaptive immune responses to foreign microbial antigens [22-24].

To mount immediate immune responses and to clear pathogens, the innate immune system has evolved to rapidly recognize conserved molecular patterns generally restricted to pathogenic microorganisms by using various pattern-recognition receptors (PRRs). Certain examples of PRRs are complement receptors, Fc receptors, mannose receptors and TLRs that have been a recent focus of intense research. Besides its pivotal role in the development of protective immune responses to foreign-antigens, accumulating evidence also suggests that the innate immune system is closely linked to the development of adaptive autoimmune responses to self-antigens in the context of an inflammatory environment that can provide signals for Antigen presenting cell (APC) maturation. APCs such as DCs and macrophages efficiently capture and process self-antigens from infected or dead cells and present them to autoreactive T cells by means of cross-presentation. If the release of self-antigens occurs in the absence of signals that promote APC maturation, these APCs remain immature and normally induce T cell tolerance by deletion or anergy [25].

However, this cross-presentation in the context of an inflammatory environment, whether infectious or noninfectious, would be able to break self tolerance by providing signals that promote APC maturation. Intriguingly, certain self-components have been revealed to act as autoantigens, not only because they provide antigenic epitope, but they also inherently have the capacity to engage PRRs such as certain Toll-like receptor (TLRs) efficiently and thereby promote APC maturation that leads to the activation of self-antigen-specific T and B cells [26].

These critical roles of innate immune system in orchestrating the adaptive immune response to self- as well as foreign-antigens raises the possibility that aberrant regulation of the innate immune system can lead to the development of autoimmune diseases by dysregulated activation of APCs and/or other components of the innate immune system [24]. In respect of the pathogenesis of T1D, it is becoming evident that defects in the innate immune system play a major role in the pathogenesis of T1D [21]. Although autoreactive T cells contribute significantly to  $\beta$ -cell death in T1D as the most important effector cells, innate immune cells including macrophages, DCs, NK, NK T and  $\gamma\delta$  T cells are important players in the initiation and progression of T1D by triggering and tuning of the anti-islet immune responses [24].

## Prevention of Type I Diabetes Mellitus: The Role of Immune Interventions

It has recently become evident that dysregulation of innate immune system can precipitate autoimmune diseases including T1D. Given its critical role in orchestrating adaptive immune responses, the innate immune system would be expected to play an important role in triggering and/or tuning autoimmunity by modulating adaptive immune responses to self-antigens [24].

In this regard, T1D could be effectively prevented by regulation of innate immune cells, which could provide a new therapeutic potential that has not been possible with modulation of adaptive immunity alone. Accumulating evidence suggests that, while some antigen-based immunotherapies have proved to be protective against the development of T1D in animal models, these protocols might not be successfully translatable to human patients at the time of diagnosis due

to the stochastic nature of pathogenic and tolerogenic antigen selection in animal models and human individuals [27].

The first immune intervention at diagnosis of type I diabetes mellitus in children and adolescents was plasmapheresis, which started at the end of the 1970s. This diagnostic test showed a positive effect on preservation of residual insulin secretion [28] in comparison with controls. The use of cyclosporin has been considered a breakthrough since it showed a significant preservation of insulin secretion [29]. However, the adverse effects were too serious to allow clinical use. Since then several other forms of immune intervention have been tried viz; immunoglobulins, azathioprine, linomide, antithymocyte globulin and prednisone, photopheresis, and antioxidants [30] albeit with too limited effect and/or too serious risks. Further, nicotinamide has also failed for prevention of type I diabetes mellitus [31].

The search for more specific immunotherapy continued. When antigen is presented to the T cells, one of the important receptors is the CD3 receptor. Monoclonal antibodies against this receptor can be expected to block or at least modulate the immune process. Studies using monoclonal anti-CD3 antibodies have shown that it is possible to block the destructive autoimmune process and thereby at least postpone the decline of beta-cell function [32,33]. Further studies are ongoing to determine if the effect can be prolonged with a booster treatment, where the initial treatment is followed by a booster treatment period six months later [30].

The anti-CD3 treatment is perhaps the most efficacious immune modulation currently although it is not specific enough to avoid side effects. Majority of patients experienced some degree of cytokine release syndrome. A number of side effects are seen in most patients, such as nausea, fever, muscle pain, thrombocytopenia with risk of bleedings, leukocytopenia with increasing frequency of infections, and anemia. It will be more difficult to justify treatment with therapies that carry substantial risk in children and adolescents. Some young patients hesitate to accept treatment because of the long and intensive treatment. Even adults hesitate to accept a treatment that carries significant risks and burden without evidence that the effect on preservation of insulin secretion is long lasting [30].

Teplizumab is a humanised, anti-CD3 monoclonal antibody that has been mutated to greatly reduce Fc receptor and complement binding [34]. In an early trial of anti-CD3 antibody [35], 24 patients with recent-onset diabetes were randomised equally to receive open-label teplizumab (34 mg cumulative dose for one 14-day course in a 70 kg individual) or no antibody for 14 days, with daily dose based on previous transplantation trials. At 12 months, C-peptide response to a mixed meal was maintained in 60% of treated patients versus 8% of controls ( $p < 0.03$ ). In a trial of otelexizumab [32], another monoclonal anti-CD3 antibody with reduced binding to the Fc receptor,  $\beta$ -cell function was preserved in patients receiving otelexizumab and their insulin needs were decreased up to 48 months after treatment. Adverse events, including Epstein-Barr virus reactivation, were more frequent than in the teplizumab trial [35], which is consistent with the higher cumulative dose [36]. A much lower dose of 3.1 mg otelexizumab was subsequently used in a phase 3 trial, but the primary efficacy outcome of change in C-peptide at month 12 was not met [37]. Further, the primary endpoints were not met. There was no benefit from a second administration of the monoclonal antibody 6 months later [38].

Autologous non-myeloablative hematopoietic stem-cell transplantation, another possible immune intervention against type

I diabetes mellitus, has been performed in 15 newly diagnosed type I diabetics aged 14-31 years [39]. Five patients were reported insulin-free after more than 21 months, and another 7 were reported insulin-free after more than 6 months. These results have to be weighed against serious adverse events (SAEs) observed in several of the patients and serious potential risks, since this type of treatment has caused acute mortality when used for other autoimmune diseases. In addition, use of such heavy cytostatic treatment including cyclophosphamide 2 g/m<sup>2</sup> body surface causes substantial risk of late adverse effects such as secondary cancer. Thus more studies in well-informed adult T1D patients are needed before this type of treatment can be regarded as ethically and clinically justified, especially in younger patients [30].

Autoantigen activation has equally been tried as an immune intervention against type I diabetes mellitus. It has long been known that exposure of specific amounts of the actual antigen can trigger immunomodulation, thereby resulting in reduction or prevention of the allergic reaction. This phenomenon partly appears to be mediated by increased T-cell regulation [WHO position paper, 1998]. Studies of animal models of autoimmune diabetes have shown that treatment with autoantigens may delay or postpone/prevent development of diabetes [30].

Parenteral insulin therapy prevents diabetes in animal models [40-45]. Moreover, pilot studies have suggested that insulin therapy also delays diabetes in humans [46-48]. Animal studies have suggested that insulin may be acting metabolically [45,49,50] — by causing the beta cells to rest [51] — or immunologically [52]. Such studies have been so convincing that many physicians have begun to use insulin in persons who are at high risk for diabetes.

A randomized, controlled clinical trial was undertaken in order to determine whether insulin could prevent or delay the onset of overt diabetes in relatives of patients with diabetes. Relatives were studied because they have a risk of diabetes that is 10 to 20 times that in the general population. The results demonstrate that insulin, in small doses, can indeed be administered safely to persons who are at risk for diabetes. A second trial studying the effect of oral insulin therapy in relatives with a projected five-year risk of 26 to 50 percent is ongoing [51].

Insulin aside, a number of agents are under development to prevent immune attack of beta cells by modulating the immune system. As a putative shared mechanism, these therapies shift the balance among the CD4<sup>+</sup> T cells from the Th1 state (characterized by “attacking” killer T cells) to the Th2 state (characterized by cytokines that inhibit inflammation). This intended Th1–Th2 shift should result in reduced proinflammatory cytokines and increased regulatory T-cells that release inhibitors of inflammation [53].

One immunomodulatory approach is a synthetic peptide sequence of an endogenous heat shock protein 60, Diaprep 277 (AndroMeda Biotech, Ness Ziona, Israel). This agent has reached phase-3 trials of adults with new-onset T1D. Treatment has been associated with significant preservation of insulin secretion and no apparent drug-related side effects [54]. However, these results remain to be confirmed in ongoing trials. Studies of children and adolescents with T1D have shown no effect, [55] which could possibly be explained by the more intense autoimmunity typically seen with diabetes onset in the younger ages. In addition, LADA patients are currently ongoing with Diaprep 277 treatment [30].

Immunomodulation with Glutamic Acid Decarboxylase (GAD) can also be an effective immune strategy against type I diabetes mellitus. Gamma-aminobutyric acid (GABA), formed on demand when glutamic acid, or glutamate, is decarboxylated by the enzyme, GAD, is one of the important neurotransmitters [30].

As GABA is an inhibitory neurotransmitter, loss of GAD activity and decrease of GABA synthesis from glutamate can result in loss of GABA-dependent signal modulation, which may lead to hyperactivity and seizures. A reduction of GABA in brain levels has been demonstrated in patients with stiff person syndrome (SPS). This syndrome is a very rare disorder characterized by muscle rigidity and episodic spasms. Anti-GAD antibodies are found in high titers in most SPS patients [56], but patients with SPS and T1D differ both in the epitope recognition and the isotype pattern of autoantibodies to GAD65 [57].

Glutamic acid decarboxylase also exists in the pancreas, but with yet unknown physiological role [30,58]. Suggestively, GABA regulates hormone release in the pancreas and/or functions as a paracrine signaling molecule for communication between the beta cells and other endocrine cells in the islets. Other studies suggest that GABA, generated by GAD65, may function as a negative regulator of first-phase insulin secretion in response to glucose [59].

Autoantibodies to GAD (GADA) may be an early sign of the autoimmune process of diabetes, and GADA has become one of the most important predictive markers of T1DM risk [60,61]. In autoimmune diabetes a T-cell response against the beta cells seems to be crucial [62-66]. T-cell reactivity to GAD65 peptide is shared with a protein of the Coxsackie virus, which itself has been implicated as an environmental trigger of T1D [67-69].

Glutamic acid decarboxylase vaccination is intended to modulate the immune system and thereby prevent the destruction of beta cells. Studies of nonobese mice with diabetes show that administration of the GAD65 isoform can prevent autoimmune destruction of beta cells. These findings suggest that GAD65 administration could be used as a preventive treatment for T1D [30].

A GAD vaccine (Diamyd®, Diamyd Medical AB, Stockholm, Sweden) with aluminum hydroxide (alum) as adjuvant has been produced and is now being investigated in phase-3 trials. Aluminum salts enhance the presentation of antigens to antigen-presenting cells. Injected GAD65 is processed by antigen-presenting cells to provide peptide fragments recognized by T cells. This results in a Th1/Th2 shift consisting of induction and proliferation of a subset of GAD65-specific regulatory T cells. These specific T cells down-regulate antigen-specific killer T cells that would otherwise attack the beta cells [30].

## Conclusion

Against this background, it is evident that the dedicated efforts towards developing an effective immunomodulatory approach to management and/or prevention of type I diabetes mellitus are possible and are working. The advances in development of more efficacious immune interventions are envisaged to play a critical role in management of type I diabetic burden in the world.

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