INTRODUCTION

T cells originate from hematopoietic stem cells in the bone marrow. The development of a T cell is shaped through positive and negative selection [1]. Low affinity interactions with self-antigens present on major histocompatibility complex (MHC) peptides provoke positive selection, whereas high affinity interactions provoke negative selection. T cells that strongly interact with the self-antigen are deleted by negative selection through apoptotic signals in the thymus, which is a major mechanism of central tolerance. T lymphocytes, include specialized subsets of regulatory T cells (Tregs) that are crucial for the maintenance of immunological tolerance [2]. Their major role is to shut down T cell-mediated immunity towards the end of an immune reaction and suppress auto-reactive T cells that escaped the process of negative selection in the thymus. Destruction of β-cells via autoimmune processes occur, when immune-regulatory mechanism fails, allowing auto reactive T cell clones to infiltrate the pancreas, thereby selectively destroying the β-cells in the islets of Langerhans [3, 4].

In general, Type 1 diabetes (T1D) develops in the young population. Restoration of insulin secretion and normalization of glucose levels are essential to lead a healthy life. Exogenous administration of insulin is quintessential and a widely used treatment for T1D. Though the discovery of insulin transformed T1D treatment, it made this a fatal and devitalizing disease. Exogenous administration of insulin in the instance of T1D is passive in nature that does not deal with the cause of the disease. Successful results using insulin therapy remains inadequate.

In fact, T1D is reversed by islet transplantation; nevertheless, it is associated with the surgical morbidity and hostile effects of persistent immune suppression [5]. The body often destroys transplanted islet cells owing to MHC of host identifying the graft tissue as foreign antigens and attacking them. Ergo, the main challenge in a successful transplantation is to find tissue types as similar as possible for both host and donor. However, availability of human islets from cadaveric pancreata is meager and dearth of islet source resulted in the quest for methods of generation of islet cells in vitro and in vivo.

The discovery of antibodies has had an enormous impact on the field of immunology. In recent years, mainstream research has been focused on the pathological significance...
of immune tolerance in T1D. Monoclonal antibodies (mAbs) are a breakthrough for
immune based therapies. They could induce tolerance/protective immunity either by
targeting the regulatory immune responses or eliminating antigen reactive clones,
which can avert autoimmune diabetes.
Prevention of autoimmunity is not only a step forward for the treatment of T1D but
could also restore β-cell mass. Glucagon-like peptide (GLP)-1 is produced from
intestinal L cells and has been shown to stimulate β-cell proliferation, neogenesis, and
also has anti-apoptotic effects on β-cells [6, 7]. However, the potential use of GLP-1 as
a possible method to regenerate pancreatic β-cells is limited due to its rapid
degradation by dipeptidyl peptidase (DPP)-IV [8]. To conquer this, GLP-1 agonists
and DPP-IV inhibitors have been investigated for the treatment of diabetes [9].

1.1. Preamble
Normally, a balance exists between type 1 helper T (Th1) cells (secrete IL-2, TNF-α/β
and IFN-γ) and type 2 helper T (Th2) cells (secrete IL-4 and IL-5), which
correspondingly involve in cellular and humoral immune reactions that are coordinated
by the CD4+ T cells. T1D is believed to be dependent on the activation of Th1 cells,
which is not sufficiently counterbalanced by Th2 cells and Tregs [10-12] (Fig. 1a).
Immune tolerance is liable for the activation of the Th1 cells. It is a condition where
Tregs are specifically unable to respond on foreign/self antigen; at the same time they
can respond to foreign/diseased antigens to which they have not been specifically made
tolerant by the natural process of self-tolerance [13]. Induction of immune tolerance to
Tregs is the possible tool for the treatment/prevention of T1D [14]. In this context, a
thought provoking strategy based on the use of biological agents that selectively
interfere with lymphocyte activation, namely anti-T cell antibodies, which can
induce/restore self tolerance to well defined β-cell antigens enmeshed as prospective
targets is at the focal point of research in T1D.
In T1D, antigen-specific immunotherapy is an enviable intent that induces immune
tolerance. The primary auto-antigen that is very likely to initiate T1D is insulin peptide
B:9-23. Peptide therapy i.e., a peptide vaccine utilizing B:9-23 or a derivative of B:9-
23, came into view as an interesting approach for the treatment of autoimmune diabetes
[15]. Studies had shown that immunization with insulin peptide B:9-23 alone may be
insufficient to reverse disease or induce long-term tolerance; however, it requires combination with an appropriate immune modulator that can enhance Treg function [16, 17]. The majority of pathogenic CD4+ T cells recognize insulin B:9–23 peptide-MHC-II complex only when the insulin peptide is bound in register 3 (R3) [18]. Intervention with mAb287 blocks binding of IAg7-B:10–23 R3 tetramers associated with T cells and inhibits T-cell responses to soluble B:9–23 peptides and NOD mice islets [18].

Nevertheless, diabetes does not develop in the absence of B cells. They participate in most autoimmune diseases through production of autoantibodies, but in T1D they likely promote disease by functioning as antigen presenting cells (APCs) [17]. The anti-CD20 mAb is used in the treatment of B-cell lymphomas. It decreases the amount of CD20+ B cells; thereby reduces the autoantibody production [19]. There are many preclinical questions to be addressed in consideration of anti-CD20 mAb therapy for the treatment of T1D. This is not possible to study in a mouse model, due to lack of antibody. To address these issues, Hu CY et al. developed a NOD mouse model expressing transgenic human CD20 (hCD20), treated with humanized anti-hCD20 mAb. Finding results the combined effect, that is decrease in effector CD4 and/or CD8 T cells and induction of Tregs [20]. Pescoätz MD and colleagues carried out a double blind study involved 87 newly diagnosed T1D patients, randomly allocated to receive four infusions of anti-CD20 mAb (rituximab) or placebo on days 1, 8, 15, and 22. The results demonstrated the partial preservation of β-cell function over a period of 1 year in patients with T1D [21]. As the effects of B cell depletion on disease associated T cell responses were not known, efforts were initiated to minimize this ambiguity. Herold KC et al. [22] compared T cell subset distributions and auto-reactivity profiles in rituximab recipients that had positive C-peptide responses with those unresponsive to treatment from the rituximab trial (NCT00279305) [21]. B cell depletion with rituximab impacted with an increase in proliferative cellular immune responses to diabetes antigens and attenuated β-cell loss [22]. The mechanism underlying this effect is not clear, owing to the fact that eventually disease and disease progression are T cell dependent [22].

Jun Shimizu et al. [23] revealed that both CD4+ and CD8+ T cells are of vital importance in the development of T1D. CD3 is a part of the T cell receptor (TCR), thus it can be found on every T cell. Strategies were postulated by using treatment with
monoclonal antibodies to CD3 that interfere with pathogenic T-cell activation in T1D. Preclinical studies of CD3 mAbs in NOD corroborated that they were effective and induced transient depletion, especially when Fe receptor non-binding CD3 antibodies were used (i.e., in mouse, F(ab')2 fragments or IgG3 CD3 antibodies, and in patients, humanized Fe-mutated CD3 antibodies) [24-29]. CD3 mAbs act on both pathogenic T cells and Tregs. The distinct effect of CD3 mAbs cause unresponsiveness or predominantly kills the Th1 cells, whereas Th2 cells may be stimulated by them [30]. In addition, CD3 mAbs induce a population of Tregs that can prevent or lead to reversal of T1D [31, 32]. Belghith M et al. [33] made efforts to understand the mechanism of CD3 epsilon-specific antibodies involved in the supremacy of Tregs in NOD Cd28-/− mice. There are two main subgroups of CD4+CD25+ Tregs: thymus-derived, naturally occurring CD4+CD25+ Tregs and peripherally induced CD4+CD25+Foxp3+ Tregs [34]. CD3 mAbs instigate TGF-β activation, thereby inducing CD4+CD25+Foxp3+ Tregs, which regain their functional capacity to regulate inflammatory responses against destructive autoimmunity in T1D [33]. Findings from previous studies elicit that CD4+CD25high Tregs act in a cytokine independent manner, therefore suggesting another type of Treg being operational in the setting up of immune regulation [35]. A subset of FoxP3+ cells present within a CD4+CD25low lymphocyte subset suppressed T cell immunity in spontaneously diabetic NOD mice in a TGF-β-dependent manner. This distinct Treg subset was evident in NOD, but not normal mice, suggesting that NOD mice may generate these adaptive Tregs in an attempt to regulate ongoing autoimmunity [35]. This result has led to clinical trials with humanized Fe-mutated anti-CD3 mAbs in humans that interfere with pathogenic T-cell activation. The outcome of this study yielded favorable clinical effects, such as reduced insulin intake, and decline of C-peptide levels [29, 36-38], which was further supported by recent clinical studies [39-42].

Deficiency in Tregs is a critical determinant of diabetes susceptibility [43]. D’Alise AM et al. [44] reported the lack of a primary deficit in Treg numbers in NOD mice, whether in prediabetic animals of any age or in those with recent-onset diabetes. The functional inefficacy in T1D was not rooted with NOD Tregs suppression but with effector conventional Th cells. Treg cell activation is antigen-specific, which implies that suppressive activities of Treg cells are antigen-dependent [45]. To substantiate
this, D’Alise AM et al. executed a study in which conventional Th cells, rather than Tregs, responded more effectively to anti-CD3/28 mAb stimulation in vitro or to a natural pancreatic antigen in vivo. This difference was independent of autoimmune inflammation, did not map to the idd3 region, and was not due to the overproduction of IL-21 in NOD mice. Thus, the immune dysregulation in this T1D model was entrenched in the ability of effector T cells to be regulated [44].

The important role of conventional Th cells, particularly Th1 cells, in pathology of T1D entailed depletion of CD4+ T cells, which initiated the use of depleting CD4 mAbs against CD4+ T cells, that interfere with induction of T1D (Fig. 1b). Bulk elimination of T cells including Tregs involved in immune regulation, may subsequently result in immune suppression. In addition, depletion of target cells may not be essential in CD4 mAb mediated immune regulation [46, 47]. Assessing this, Shixin et al. and Hutchings et al. used non-depleting mAbs contrary to CD4 mAbs, and reported that a short course of non-depleting mAbs enabled a rapid response on proficient cells involved in autoimmune destruction of β-cells [46, 47]. Adding to it, non-depleting CD4 mAbs could alleviate the risk of immune suppression by interrupting the elimination of Tregs.

The ultimate goal of therapeutic intervention is to not only shut down the autoimmune responses hostile to β-cells, but also to enable the restoration of endogenous insulin secretion and normalization of glucose levels. Thus, finding means to restore β-cell mass, by initiating their replication or regeneration from precursor cells is a major goal of current research.

It is a widely known fact that ghrelin receptor hormone GLP-1 can act as a growth and differentiation factor for mature β-cells and their precursors [6-9]. GLP-1 receptor stimulation leads to the activation of adenylate cyclase with the help of G-proteins. Activated adenylate cyclase forms cAMP, which in turn activates protein kinase (PK)-A and eventually translocates to the nucleus through the nucleus pore. These activated PK-A, phosphorylate the cAMP response element binding (CREB) protein. Phosphorylated CREB binds to a cAMP response element region (CRE), subsequently binding to CREB-binding protein (CBP), thereby coactivating it and allowing it to express various genes and proteins. Embracing this mechanism in the current context may pave the way to achieve the therapeutic goal of restoring functional β-cells through the mitogenic action of GLP-1 on the β-cell [6].
Despite, GLP-1 being a feasible avenue to regenerate pancreatic β-cells, DPP-IV invariably breaks it down. Previous reports suggest that a sustained treatment of exogenously administered GLP-1 is required to influence β-cell regulation [8]. DPP-IV inhibition has come into view as an interesting preventive therapeutic approach. As the correlation between DPP-IV inhibition and β-cell neogenesis is well established [48], this effect is believed to be predominantly as a result of longevity of the incretin hormones including GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) (Fig. 1c). Till date, a majority of studies performed on animal models sought to reveal the role of DPP-IV inhibitors in the treatment of T1D [48, 49]. Results from these studies corroborate that inhibition of DPP-IV may ameliorate the β-cell mass and improve functional aftermath. Ellis et al. [50] for the first time evaluated the clinical efficacy of sitagliptin on glucose control and variability in adult patients with T1D. Sitagliptin significantly improved overall glucose control including postprandial and 24h glucose control, while significantly reducing prandial insulin requirements in adult patients with T1D. These findings suggest that therapeutic potential of DPP-IV inhibitors may extend beyond glycemic control to include augmentation of β-cell mass and function. In addition, Kim et al. [51] studied the effects of sitagliptin (a DPP-IV inhibitor) on CD4+ T cell migration in NOD mice as DPP-IV increases migration of splenic CD4+ T cells via a pathway involving cAMP/PK-A/Ras-related C3 botulinum toxin substrate 1 (Rac1) GTP binding activity. Results portrayed the inhibitory potential of sitagliptin towards the migration of splenic CD4+ T cells.
1.2. Hypothesis

Treatment with CD4 mAbs conjugated sitagliptin-loaded polymeric nanoparticulate drug delivery system, thereby selective depletion of CD4⁺ T cells and restoration of the β-cell mass would be a new therapeutic approach for the correction of T1D.

![Diagram](image)

**Fig. 1. Consequential strategy of our hypotheses.** Schematic representation of (a) pancreatic β-cells autoimmune destruction. The CD4⁺ T cells secrete cytokines such as IFN-γ, TNF-α/β, and IL-2, which may activate β-cell specific pre-cytotoxic T cells (CD8⁺ T cell) to differentiate into effector T cells. IFN-γ released by CD4⁺ Th1 cells may activate macrophages to release substantial amounts of β-cell cytotoxic cytokines. Cytokines and granzymes/perforin, which are toxic to β-cells released from macrophages and CD8⁺ T cells, respectively, may induce the destruction of pancreatic β-cells. Oxygen free radicals secreted from activated macrophages can also kill β-cells. (b) Role of Th1 cells in pathology of T1D entailed depletion of CD4⁺ T cells, which initiated the use of CD4 mAbs against CD4⁺ T cells that interfere with induction of T1D, and (c) DPP-IV inhibition may lead to longevity of GLP-1 half-life which in turn may augment the β-cells mass through cAMP/PKA/CREB pathway.
1.3. Implications of the hypothesis

Our hypothesis reinforced the certainty that T1D results from the autoimmune destruction of the β-cells by auto-reactive T cells [1-4]. Sherry et al. [52] divulged the efficacy of exendin-4 in combination with CD3 mAbs in NOD mice, which showed improved β-cell mass, and subsequent increase in insulin secretion. Earlier reports recommended the use of CD4 mAbs as efficacious and less toxic immunosuppressive agents than CD3 mAbs [53]. Extension of the GLP-1 half-life using long acting GLP-1 analogues would be insufficient to regenerate β-cells for the control of T1D, unless these compounds are administered multiple times over each day. Polymeric nanoparticles have recently attracted great attention as potential drug delivery systems in view of their applications in the sustained release of drugs. Due to their nano size, the particles penetrate into small capillaries and are taken up within cells, allowing an efficient drug accumulation at the targeted sites in the body. The use of biodegradable materials for nanoparticles preparation allows sustained drug release at the targeted site over a period of days or even weeks [54]. Hence, our investigation aspires to determine if extending the shelf life of GLP-1 through sitagliptin loaded polymeric nanoparticles would regenerate pancreatic β-cells. In addition to this, CD4 mAbs conjugated to sitagliptin loaded polymeric nanoparticles could provide better results, the reason being that CD4+ T cells play a central role in T1D. CD4 mAbs selectively deplete CD4+ T cells, and at some extent sitagliptin has selective effects on subpopulation of T-cells that are involved in the autoimmunity [51].

In conclusion, CD4 mAbs could arrest the autoimmunity in T1D and sitagliptin loaded polymeric nanoparticles could regenerate pancreatic β-cells. Altogether, antibody-drug conjugates could avoid or at least minimize the constraints of intensive subcutaneous insulin therapy. Thus, consequential strategy of our hypothesis may be an efficacious approach for the treatment of T1D. The design of our hypothesis includes the development of CD4 mAbs conjugated sitagliptin loaded polymeric nanoparticulate drug delivery system and evaluation of its efficiency in vivo; the feasibility of our hypothesis is encompassed in this.