REVIEW

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# Immunotherapy of type 1 diabetes

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## Abstract

Type 1 diabetes (T1D) is an autoimmune disease in which the insulin-producing β cells are destroyed. Diabetic patients manage their hyperglycemia by daily insulin injections. However, insulin therapy is by no means a cure. Accordingly, a significant effort has been ongoing to develop immunotherapies that effectively prevent and/or treat T1D in the clinic. This review focuses on antigen- and antibody-based immunotherapies and discusses the respective strengths and weaknesses of these approaches.

**Key words:** antibody, antigen-specific, immunoregulatory T cells, tolerance.

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## INTRODUCTION

Type 1 diabetes (T1D) is characterized by the autoimmune-mediated destruction of the insulin-producing β cells of the pancreatic islets of Langerhans [5, 9, 36, 108]. The disease affects approximately 1 out of 250 individuals in North America, mostly children and adolescents, although both the incidence and age at onset are increasing. Despite daily insulin injections, diabetic individuals typically develop complications associated with aberrant glucose metabolism, including retinopathy, neuropathy, cardiovascular disease, and nephropathy. Consequently, there is a need for effective modalities to prevent and/or treat T1D.

Failure in self tolerance towards β cells involves a series of complex events that are governed by environmental and genetic factors. T1D is initiated and/or progresses when genetically predisposed individuals encounter an environmental insult [5, 9, 36, 108]. More than 20 chromosomal loci contribute to T1D susceptibility, with the most prominent genes identified to date encoding specific alleles of major histocompatibility complex (MHC) class II molecules [113, 114, 123], insulin [8], and CTLA-4 [59, 115]. Environmental agents influencing T1D susceptibility remain largely ill-defined, although microbial infections and diet have been implicated [5, 9, 36, 108]. Studies using rodent models of spontaneous T1D, such as the nonobese diabetic (NOD) mouse, have demonstrated that $CD4^+$ and $CD8^+$ T cells are the primary mediators of β-cell destruction, although other immune cells, including B cells, NKT cells, dendritic cells (DCs), and macrophages are also involved in the development of diabetes [5, 9, 36, 108]. Pathogenic $CD4^+$ and $CD8^+$ T cells typically exhibit a type 1 phenotype and target a select panel of β-cell autoantigens and epitopes at early stages of preclinical T1D [65, 77, 112]. As β-cell autoimmunity progresses, additional intra- and intermolecular epitopes are recognized, thereby amplifying the pathogenic T-cell response [116]. The existence of β cell-reactive T cells in healthy individuals and the apparent skewing towards the differentiation of type 1 T effectors in diabetic individuals and NOD mice provide indirect evidence that defective peripheral immunoregulation contributes to β-cell autoimmunity [5, 7, 9, 36, 108]. Indeed, a reduced frequency and/or aberrant function of immunoregulatory T effectors such as FoxP3-expressing $CD4^+CD25^+$ Treg cells and IL-4- and IL-10-secreting Th2 and Tr1 cells, respectively, has been reported in NOD mice and diabetic patients [7, 19, 40, 47, 55, 70, 86]. Accordingly, immunotherapies have focused on reestablishing the functional balance between pathogenic T cells and immunoregulatory effectors in order to block the progression of β-cell autoimmunity.

## APPROACHES OF IMMUNOTHERAPY FOR T1D

β-Cell autoimmunity is marked by the progressive infiltration of the islets, termed insulitis. Evidence from the NOD mouse indicates that insulitis undergoes qualitative changes in a temporal manner [6]. Early insulitis is relatively "benign"; however, at later stages an "aggressive" form of insulitis develops at which time β cells are efficiently destroyed. Avidity maturation and resistance to suppression by pathogenic T effectors [4, 47], coupled with aberrant immunoregulation, are thought to contribute to the qualitative change in insulitis. Once a sufficient amount of β-cell mass has been destroyed (~85–90%), overt diabetes is clinically diagnosed. Two general scenarios exist for the immunotherapy of T1D. The first is to prevent the onset of diabetes in individuals at risk. Ongoing β-cell autoimmunity in at-risk individuals can be monitored by detection of serum autoantibodies specific to various autoantigens such as insulin, glutamic acid decarboxylase 65 (GAD65), and insulinoma-associated tyrosine phosphatase (IA-2) [36]. The second scenario of T1D immunotherapy is to treat individuals at the onset of diabetes. A significant amount of β-cell mass remains at the time diabetes is diagnosed and rescue of residual β cells has a therapeutic benefit reflected by increased control of blood glucose levels and reduced doses of insulin [94, 124]. Furthermore, suppressing autoimmunity may facilitate β-cell regeneration and/or replication [79] and under ideal circumstances induce remission of diabetes [26].

Successful treatment in either of these scenarios is governed by the degree ongoing β-cell autoimmunity can be suppressed. At late preclinical stages or at the onset of diabetes, a relatively high frequency of pathogenic T effectors exists and a proinflammatory milieu in the islets is well established. Consequently, the conditions to suppress ongoing β-cell autoimmunity become increasingly stringent as the diabetogenic response progresses. Specificity is another key factor in considering the application of a given immunotherapy. For instance, immunosuppressive drugs such as cyclosporine have been used to suppress β-cell autoimmunity in diabetic individuals [16]. However, due to the general immunosuppressive (and toxic) effects of such drugs, treatment cannot be given long-term. Accordingly, a major challenge within the field is the development of immunotherapies that selectively target the pathogenic effectors and promote long-term β-cell tolerance, all the while maintaining "normal" immune function.

Several approaches of immunotherapy are being developed and tested to prevent and/or treat T1D. For the purpose of this review we will focus on two approaches, namely antibody- and antigen-based immunotherapies for which both clinical and experimental findings are available. A recent review by Staeva-Vieira et al. [101] provides an extensive listing of additional strategies and targets being tested and/or considered for the treatment of T1D.

## ANTIBODY-BASED IMMUNOTHERAPY

Monoclonal antibodies have been used to target a wide spectrum of immune components ranging from soluble mediators, such as cytokines and chemokines, to different types of cells including T cells and antigen-presenting cells (APCs) [54]. Not surprisingly, most studies in T1D have used antibodies that target $CD4^+$ and $CD8^+$ T cells either directly or indirectly. However, more recent studies suggest that B cells may also be a relevant target to alter the progression of β-cell autoimmunity.

### *Targeting T cells with antibodies*

The immunotherapeutic potency of monoclonal antibodies targeting T cells has been reported in several experimental models [29, 54]. For instance, treatment of diabetic NOD mice with short courses of depleting anti-CD4 antibody [22, 83, 96, 120] or anti-lymphocyte serum [72, 81, 97] has been effective in suppressing ongoing β--cell autoimmunity, and in some cases leading to remission in recent-onset diabetic animals. However, these and other antibodies indiscriminately deplete pathogenic and non-autoimmune T cells to induce a state of immunosuppression for significant periods of time. Furthermore, the number of T cells that reappear after the antibody has been cleared *in vivo* is usually reduced compared to normal T-cell levels [54]. In this regard, the use of nondepleting anti-CD4 and -CD8 antibodies may prove to be an effective alternative strategy. Work by Waldmann and colleagues has shown that nondepleting anti-CD4 and -CD8 antibodies in mice induce long-term tolerance to allogeneic skin grafts in an antigen-specific manner without significantly altering T-cell numbers [12, 43, 88, 118]. Furthermore, nondepleting anti-CD4 and -CD8 antibodies have been shown to prevent β-cell autoimmunity in NOD mice [30, 50, 64, 84, 119]. Binding of nondepleting anti-CD4 and -CD8 antibodies has no apparent effect on naïve T cells, but induces apoptosis in activated T cells (e.g. pathogenic effector T cells) [84]. In addition, nondepleting anti-CD4 antibody treatment induces/expands FoxP3-expressing $CD4^+CD25^+$ Treg cells, which suppress the differentiation and effector function of pathogenic T cells, a mechanism referred to as "infectious tolerance" [12, 43–45, 118]. Interestingly, binding of anti-CD45 antibody to human $CD4^+$ T cells *in vitro* also induces differentiation of an immunoregulatory phenotype characterized by IL-10 secretion [48].

Studies investigating the efficacy of anti-CD3 antibodies have generated a great deal of interest within the T1D field. Early work by Chatenoud et al. [23–26] demonstrated that treatment with a short course of low--dose anti-CD3 antibody induces remission in recent-onset diabetic NOD mice that remain free of recurrent diabetes long-term. Furthermore, tolerance is β cell--specific in that anti-CD3 antibody-treated NOD mice continue to reject allogeneic skin grafts [26]. Induction

of remission correlates with the rapid depletion of T cells infiltrating the islets. Here, anti-CD3 antibody binding preferentially affects activated versus naïve T cells by promoting: 1) down-regulation of T cell receptor (TCR), 2) reduced TCR signaling, 3) enhanced apoptosis, and 4) altered trafficking [24, 99, 100]. Notably, protection is also dependent on the induction of $CD4^+CD25^+$ Treg cells, which mediate suppression in a TGFβ-dependent manner [11, 128]. $CD4^+CD25^+$ Tregs, which are relatively resistant to the tolergenic effects of anti-CD3 antibody binding, maintain tolerance long-term by blocking the differentiation and activity of pathogenic β cell-specific $CD4^+$ and $CD8^+$ T cells.

Recent clinical studies have yielded promising results with "nonmitogenic" anti-CD3 (anti-nmCD3) antibodies [56–58]. The Fc region of these anti-nmCD3 antibodies has been engineered to enhance efficacy and safety [3, 14, 125]. Herold and colleagues treated patients within six weeks of diagnosis with a single course of anti-nmCD3 antibody and monitored β-cell function by measuring C-peptide responses over two years [57, 58]. Notably, preservation of residual β-cell function was observed in the majority of anti-nmCD3 antibody-treated patients relative to the untreated control group. This therapeutic effect correlated with a transient increase in IL-10 secreting T cells and FoxP3-expressing $CD8^+CD25^+$ T cells. In a subsequent clinical trial, Chatenoud's group demonstrated a significant protective effect with anti-nmCD3 antibody in a subset of patients that displayed increased β-cell function at the time of treatment [66]. This subset may represent patients at an earlier stage of disease progression, suggesting that the timing of anti-nmCD3 antibody is critical in order to elicit a therapeutic effect. Administration of anti-nmCD3 antibody results in a transient decrease in T cells in blood and is generally well-tolerated. However, some patients present with "flu-like" symptoms due to significant cytokine release by antibody-bound T cells, and recurrent Epstein-Barr viral infections are also detected [57, 58, 66]. Although efficacy was seen in these trials, the protection mediated by anti-nmCD3 antibody treatment was nevertheless transient. Therefore, the dose, intervals, and/or route of anti-nmCD3 antibody treatment may have to be modified to enhance efficacy and minimize unwanted complications.

Other antibody-based approaches to modulate β cell-specific T-cell reactivity may involve targeting APCs such as DCs and B cells. For instance, blocking the CD40-CD40L axis has proven to be highly effective in blunting pathogenic T-cell responses in autoimmune and transplantation models [35, 41, 61, 73]. Although anti-CD40L antibody has been discontinued in clinical trials due to adverse platelet aggregation, application of antibodies that block CD40 may circumvent this problem in patients. Using antibodies or antagonists specific to relevant chemokines and adhesion molecules may block T-cell recruitment into the pancreatic lymph nodes (PLNs), where T-cell priming occurs, or in the islets. This approach, however, may be more effective early in disease progression or after islets have been depleted of T cells by anti-CD3 antibody treatment, for instance.

*Targeting B cells with antibodies*

The primary role of B cells in T1D is to serve as APCs for T cells. Islet-specific autoantibodies are generally thought to have only a minimal (if any) role in β--cell destruction. Consequently, targeting B cells may provide an indirect approach to manipulate β cell-specific T-cell reactivity [17]. Indeed, two recent studies in the NOD mouse provide support for this strategy [63, 126]. In the first, B cells were depleted with a short course of monoclonal anti-mouse CD20 antibody in young NOD mice or in older euglycemic animals in which β-cell autoimmunity was well established [126]. Despite the reappearance of B cells, the majority of NOD mice treated at a young age remained diabetes--free long-term. On the other hand, anti-CD20 antibody treatment was less effective in the older treated NOD mice, in which a significant delay in the onset of diabetes was observed. In the second study, NOD mice transgenic for a human CD20 molecule were treated with a human CD20-specific antibody (2H7) [63]. 2H7 recognizes the same epitope as Rituximab, which has been used in the clinic to deplete $CD20^+$ non-Hodgkin's B lymphoma cells and has been tested in patients with rheumatoid arthritis [42], systemic lupus erythematosus [67], and multiple sclerosis with encouraging results [32]. A significant delay in the onset and a reduced frequency of diabetes was detected in NOD.hCD20 transgenic mice treated with 2H7. Interestingly, remission was detected in ~30% of recent-onset diabetic NOD. hCD20 transgenic mice treated with the antibody. Furthermore, 2H7-induced protection correlated with the expansion of $CD4^+CD25^+Foxp3^+$ and CTLA-$4^+$ T cells and a reduced T cell-stimulatory capacity of macrophages and splenic DCs. Moreover, B cells from antibody-treated mice also appeared to exhibit regulatory function. Differences between the two studies may reflect the distinct models and/or specificity and binding properties of the respective antibodies. Nevertheless, these studies indicate that β-cell autoimmunity can be altered by targeting B cells and provide support for an ongoing phase II clinical trial in which recent-onset diabetic patients are treated with Rituximab.

## ANTIGEN-BASED IMMUNOTHERAPY

Antigen-based immunotherapy is an approach to selectively target disease-relevant T cells and maintain the "normal" function of the immune system [39, 109]. Numerous studies have demonstrated that antigen--based immunotherapy can be highly effective in preventing and suppressing autoimmunity in rodent models

of T1D and other autoimmune diseases [51, 52, 65, 74, 112]. Successful application of antigen-based immunotherapy in the clinic, on the other hand, has yet to be achieved. The Diabetes Prevention Trial-1 (DPT-1), in which insulin was administered either parentally or orally in prediabetic subjects, demonstrated no significant effect on the development of diabetes or β-cell autoimmunity [1, 98]. The lack of efficacy in the majority of subjects was partly attributed to administration of an insufficient dose of insulin. Interestingly, a treatment effect was detected in a subset of patients receiving oral insulin who presented with high titers of insulin autoantibodies [98]. Therefore, the degree of existing autoimmunity for a given self antigen may be a key determinant for the induction of an immunotherapeutic effect.

Treatment with self antigen can affect autoreactive T cells in two mutually nonexclusive ways, namely the induction of T-cell anergy/deletion and the establishment of immunoregulatory T cells [69, 109, 122]. Administration of high doses of soluble antigen induces T-cell anergy and/or deletion, which can be effective if there is a single or dominant autoantigen [10, 34]. However, when multiple autoantigens are targeted, as seen in the late preclinical and clinical stages of T1D, the efficacy of anergizing or deleting a single or few clonotypes is limited. Consequently, the induction/ /expansion of immunoregulatory T cells is the favored approach when multiple specificities are recognized in the disease process. Once established, immunoregulatory T cells modulate responses in a bystander fashion via cytokine secretion, for example, independently of the specificity of the autoreactive T cells. In addition, suppression of the proinflammatory milieu promotes the further differentiation of immunoregulatory T cells with varying specificities to amplify the tolergenic response.

Naïve β cell-specific $CD4^+$ T cells can be induced to differentiate into a variety of "adaptive" immunoregulatory effectors which include: 1) IL-4-secreting Th2 cells, 2) IL-10-secreting T cells, and 3) adaptive $CD4^+CD25^+$ Treg cells in which FoxP3 and TGFβ expression are up--regulated [27, 28, 49, 62, 107]. Under certain conditions, β cell-specific "natural" $FoxP3^+$ $CD4^+CD25^+$ Tregs, which are characterized by a suppressor function that is established in the thymus, may also be expanded in the periphery [104, 127]. It is noteworthy that these subsets of $CD4^+$ T cells exhibit distinct potencies to immunoregulate autoimmune responses. For example, natural and adaptive FoxP3-expressing $CD4^+CD25^+$ Tregs exhibit the most potent suppressor function, regulating naïve and effector $CD4^+$ and $CD8^+$ T cells, B cells, and APCs via cell-cell contact and/or expression of TGFβ [93]. IL-10-secreting $CD4^+$ T cells, which consist of a number of subsets, are also noted for a potent immunoregulatory effector function. IL-10 not only affects $CD4^+$ and $CD8^+$ T-cell reactivity directly, but also indirectly by inhibiting activation of immature DCs and promoting a "tolergenic phenotype" [49, 80, 92].

Several factors are at play determining the efficacy of an antigen-based immunotherapy, and specifically the induction and/or expansion of immunoregulatory T cells. One key factor is the number of immunoregulatory T cells that are induced upon vaccination with a given autoantigen. At late preclinical and clinical stages of T1D a relatively high frequency of immunoregulatory T effectors is needed to effectively suppress robust β-cell autoimmunity. The number of adaptive immunoregulatory T effectors is dependent on the size of the pool of naive precursors for a given β-cell autoantigen [82, 106, 110]. Therefore the choice of autoantigen used for treatment at late stages of disease progression is critical. Specific clonotypes of T cells actively involved in β-cell destruction would be expected to have only a limited pool of naive precursors, therefore minimizing the number of potential immunoregulatory T effectors that can be induced.

In NOD mice, administration of a select panel of β--cell autoantigens (and derived peptides) consisting of insulin, GAD65, and heat shock protein 60 (HSP60) suppress β-cell autoimmunity at late preclinical and clinical stages under the appropriate conditions [33, 37, 38, 65, 87, 110, 111]. However, β cell-specific T-cell reactivity in patients is largely ill-defined. Only recently have approaches such as HLA class II tetramers and sufficiently sensitive ELISPOT assays been established to detect low-frequency β cell-specific T cells in human blood [76, 91]. Notably Arif et al. [7] have identified peptides derived from proinsulin, GAD65, and IA-2 that *in vitro* elicit an increased frequency of IL-10--secreting $CD4^+$ T cells from HLA-matched nondiabetic versus diabetic subjects. Vaccination with these peptides may preferentially induce IL-10-secreting immunoregulatory T cells *in vivo*. Injection of the human HSP60--derived peptide p277 (DiaPep277) to recent-onset diabetic adults in a phase II trial resulted in the induction of an increased frequency of HSP60-specific T cells secreting IL-10 and preservation of C-peptide responses over a 20-month period [90]. One potential issue highlighted by the DPT-1 is that the magnitude of the response to and, in turn, efficacy associated with a given β-cell autoantigen(s) (or peptide(s)) may significantly vary from one individual to another due to marked differences in the repertoire of β cell-specific T precursors. In this instance, it may be necessary to vaccinate with multiple autoantigens (or peptides). As additional clinical studies are carried out, better insight will be gained into which β-cell autoantigens provide the most appropriate targets to induce immunoregulatory T effectors, in addition to the variability of induced responses among subjects.

Another key factor influencing the success of an antigen-based immunotherapy is the efficiency of immunoregulatory T-cell induction and/or expansion, especially under *in vivo* conditions that favor a pro--inflammatory or type 1-mediated cell response. The latter may in fact promote the development of pathogenic effector T cells following vaccination with a self antigen [121]. A number of strategies have been tested to preferentially promote the induction and/or expansion of

immunoregulatory T cells. Exploiting the intrinsic properties of mucosal tissues to induce immunoregulatory T cells is one such strategy. Administration of antigen via nasal or oral routes has been effective in inducing IL-10- and TGFβ-secreting $CD4^+$ T cells and preventing diabetes in NOD mice [13, 60, 105]. Furthermore, the Harrison group demonstrated that intranasal administration to prediabetic individuals reduces insulin-specific type 1 T effector cell reactivity [53]. Currently, phase I and II trials have been initiated further testing the efficacy of intranasally delivered insulin. Various types of adjuvants have also been employed to promote differentiation of immunoregulatory T effectors. Alum is an adjuvant with well-established properties that promote type 2 immunoregulatory T-cell differentiation [89]. A phase I/II clinical trial in which latent autoimmune diabetes in adult patients were treated with human recombinant GAD65 formulated in alum showed no adverse effects and an improvement in C-peptide responses [2]. Cholera toxin B is an adjuvant used to enhance the efficacy of orally delivered antigen. For instance, the protective effect of oral insulin in NOD mice was increased by cholera toxin B, which in turn correlated with the induction of IL-10-secreting insulin--specific $CD4^+$ T cells [85]. Co-administration of cytokines with antigen is another approach to selectively "tailor" the nature of the T-cell response. The efficacy of plasmid DNA (pDNA) vaccines encoding GAD65 and insulin B chain is significantly enhanced in prediabetic and diabetic NOD mice by co-administration of IL-4- and IL-10-producing pDNAs [31, 87, 111, 121]. Manipulating the "context" of the antigen can also be an effective strategy to increase the efficiency of immunoregulatory T-cell induction. Linking peptides to an IgFc fragment or soluble MHC class II-Ig dimer molecule (sMHCIg-peptide) significantly enhances the immunotherapeutic capacity of the peptide [20, 46, 71]. Both strategies increase the *in vivo* half-life of the peptide and preferentially induce peptide-specific IL-10--secreting $CD4^+$ T cells, albeit via distinct mechanisms. Binding of the peptide-IgFc chimeric molecules to Fcγ receptor facilitates efficient uptake and IL-10 secretion by APCs [68]. On the other hand, direct binding of sMHCIg-peptide dimers to activated and naïve $CD4^+$ T cells induces apoptosis and the differentiation of IL--10-secreting T effectors, respectively [20, 71]. Bonifaz et al. [15] have employed complexes consisting of peptide coupled to anti-CD205 antibody in order to selectively target antigen to and exploit the tolergenic properties of immature DCs *in vivo*. CD205 is an endocytosis receptor exclusively expressed by DCs and, upon binding, the antibody-peptide complex is efficiently endocytosed and the peptide subsequently presented by the immature DCs [103]. Transgenic β cell-specific T cells adoptively transferred into NOD recipient mice are deleted by treatment with the anti-CD205 antibody complexed with the corresponding peptide [75]. The Bluestone group recently demonstrated that administration of ethylene carbodiimide-treated APCs coupled with insulin induces remission in recent-onset diabetic NOD mice [38]. The potent immunotherapeutic effect of the insulin-coupled APCs was due to anergy induction of insulin-specific T cells coupled with an increase in the frequency of FoxP3-expressing $CD4^+CD25^+$ Tregs via PD-1/PD-L1-dependent events.

Finally, other factors influencing the efficacy of antigen-specific immunotherapy include the maintenance and "variety" of induced immunoregulatory T effectors. Long-term tolerance is dependent on the persistence of sufficient numbers of immunoregulatory T cells, and maintenance of these numbers may require antigen "boosts" [87]. Accordingly, the frequency of antigen administration is very likely to be a critical parameter governing the length of protection. Whether an immunotherapy elicits a narrow versus broad set of immunoregulatory T subsets also impacts efficacy, especially at late preclinical and clinical stages of T1D [87]. A more robust tolergenic effect is mediated if a variety of immunoregulatory T-cell subsets are induced which exhibit distinct but complementary effector mechanisms.

## COMBINATORIAL IMMUNOTHERAPY

Antibody- and antigen-based immunotherapies have notable strengths and limitations. Administration of antibody allows targeting of large numbers of effector cells, although the approach is limited by the lack of discrimination between non-autoreactive and autoreactive T (and B) cells. Furthermore, long-term treatment may elicit "neutralizing" antibodies, thereby compromising the efficacy of the injected antibody. Antigen--based immunotherapies, on the other hand, can be used to selectively modulate autoimmunity. However, identification of the appropriate autoantigen(s) and whether a sufficient number of immunoregulatory but not pathogenic T effectors can be induced to suppress ongoing inflammation are key concerns. With this in mind, efforts have recently focused on combining antibody- and antigen-based approaches to exploit the respective strengths and overcome the limitations of the two strategies. Work by Bresson et al. [18] provides evidence that this approach can in fact be effective. In this study, a sub-optimal dose of anti-nmCD3 coupled with intranasal administration of preinsulin peptide was found to be more effective in inducing remission in diabetic NOD and TCR transgenic mice than either approach alone. Remission correlated with an increase in FoxP3-expressing $CD4^+CD25^+$ Treg cells, in addition to preinsulin-specific $CD4^+$ T cells secreting IL-4, IL-10, and TGFβ. Here it is believed that anti-nmCD3 antibody treatment sufficiently blocks the effector function of pathogenic T cells, rapidly rescuing β-cell mass. "Quenching" of the proinflammatory response subsequently establishes an extracellular milieu that is conducive to the differentiation and expansion of preinsulin-specific immunoregulatory T cells. Since the fre-

quency of pathogenic T effectors is reduced by anti--nmCD3 antibody treatment, fewer preinsulin-specific immunoregulatory T cells are required to suppress the diabetogenic response. Furthermore, these immunoregulatory T cells are expected to maintain β-cell tolerance long-term by "seeding" the PLNs and islets. Importantly, the dose of anti-nmCD3 antibody is reduced, thereby minimizing the likelihood of the complications associated with the antibody treatment.

A combinatorial approach can be taken to diversify the types of immunoregulatory cells and exploit different effector functions. For instance, one approach would be to combine β-cell antigen vaccination with the activation and expansion of NKT cells. Work by various groups has shown that *in vivo* activation of NKT cells by α-galactosylceramide treatment effectively suppresses β-cell autoimmunity in late preclinical T1D and at the onset of overt diabetes in NOD mice [78, 95]. The protection mediated by activated NKT cells in NOD mice is thought to be due in part to expansion of tolergenic DCs in the PLNs [78]. The latter in combination with antigen administration could result in robust induction of β cell--specific immunoregulatory T cells.

## CONCLUDING REMARKS

A number of factors impact how β-cell autoimmunity can be manipulated by immune intervention. For instance, the requirements are distinct for suppressing the diabetogenic response at early versus late preclinical stages and once overt diabetes has been established. Furthermore, emerging clinical data suggest that multiple subtypes of T1D exist which may be defined by particular genotypes and environmental exposures [21, 102, 117]. These levels of complexity may therefore necessitate the use of a number of immunotherapeutic approaches of which the most appropriate can be applied for a given clinical setting. Within this "tool box" of immunotherapies, combinatorial approaches will likely prove to be the most effective by both exploiting key strengths of the respective approaches and limiting potential adverse effects. Despite significant efforts over the last decade, the treatment of T1D in the clinic is still relatively early in the developmental process. To expedite this process, it is critical that more insight into the immunopathogenesis of human T1D be gained and assay systems to monitor β cell-specific T-cell reactivity and other immune events in patients be improved. The challenge is significant, but there is well-founded optimism that effective, long-lasting, and safe immunotherapies can indeed be developed.

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