REVIEW

Inducing immune tolerance: a focus on Type 1 diabetes mellitus

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Practice Points

- Targeting of peripheral tolerance pathways *in vivo* can induce immunological tolerance to prevent and treat Type 1 diabetes.
- Immune tolerance can be induced in an antigen (Ag)-specific manner and suppress autoimmunity without the use of broad-based immunosuppressive agents.
- Tolerance can be achieved by inducing or targeting tolerogenic dendritic cells, modulation of costimulatory and coinhibitory molecules on autoreactive T cells and induction and/or expansion of Treg cells.
- Tolerance is seen in nonobese diabetic mice, despite the many inherited defects in central and peripheral tolerance, suggesting that tolerance-based therapies may be efficacious in human patients with homologous defects that predispose them to being susceptible to autoimmunity.
- The use of high-affinity mimotopes and altered peptide ligand forms of target auto-Ags can increase the efficacy of treatment by increasing MHC presentation and MHC–T-cell interactions.
- Targeted delivery of Ags on inert particles, such as polystyrene or poly(lactic-*co*-glycolic) acid nanospheres, DEC205 fusion antibodies or tetramer complexes, can increase the efficacy of tolerance induction and can be readily translated in a clinical setting.

SUMMARY Tolerogenic strategies that specifically target diabetogenic immune cells in the absence of complications of immunosuppression are the desired treatment for the prevention or even reversal of Type 1 diabetes (T1D). Antigen (Ag)-based therapies must not only suppress disease-initiating diabetogenic T cells that are already activated, but, more importantly, prevent activation of naive auto-Ag-specific T cells that may become autoreactive through epitope spreading as a result of Ag liberation from damaged islet cells. Therefore, identification of auto-Ags relevant to T1D initiation and progression is critical to the design of effective Ag-specific therapies. Animal models of T1D have been successfully employed to identify potential diabetogenic Ags, and have further facilitated translation of Ag-specific tolerance strategies into human clinical trials. In this review, we highlight

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important advances using animal models in Ag-specific T1D immunotherapies, and the application of the preclinical findings to human subjects. We provide an up-to-date overview of the strengths and weaknesses of various tolerance-inducing strategies, including infusion of soluble Ags/peptides by various routes of delivery, genetic vaccinations, cell- and inert particle-based tolerogenic approaches, and various other strategies that target distinct tolerance-inducing pathways.

Type 1 diabetes (T1D) is a chronic autoimmune disease mediated by selective destruction of pancreatic β cells by $CD4^+$ and $CD8^+$ T lymphocytes [1–3]. Much of our knowledge of T1D disease pathogenesis and regulation derives from studies of the spontaneous murine model of T1D, the nonobese diabetic (NOD) mouse [4,5]. Additionally, T cells specific for many of the diabetogenic antigens (Ags) targeted in NOD mice have also been found in the islets and the circulation of T1D patients. The major auto-Ags include: GAD65, insulin, proinsulin, HSP60, IA-2, ZnT8 and IGRP, and are, therefore, potential primary therapeutic targets [1,6–12]. Following initial immune-mediated pancreatic damage, release of islet Ags results in epitope spreading, which leads to tissue infiltration of an increasingly diverse population of autoreactive T cells [13,14]. Thus, effective attenuation of islet-specific autoreactive T cells during the early prediabetic stage of T1D is considered an ideal therapeutic option.

Existing therapies for T1D consist mainly of insulin replacement therapy and protective therapies, which attempt to regulate the immune responses in nonspecific ways and/or promote β-cell protection/regeneration, neither of which address the underlying autoimmune pathogenesis [15,16]. The side effects and unsustainable efficacy of general immunosuppression call for improved therapies that specifically block the deleterious effects of self-reactive immune cells, while leaving the remainder of the immune system intact. This review focuses on Ag-specific tolerance strategies for the prevention and treatment of T1D and provides an overview, ranging from animal models of the disease to attempt to translate tolerance strategies, to treatment of T1D patients. The following approaches will be discussed in this article: soluble Ag-based therapies, DNA vaccines, cell-mediated tolerance, nanoparticle-facilitated tolerance and tetramer-based treatments **(Figure 1)**.

Tolerance induced by soluble Ags

NOD mice and a T-cell receptor transgenic strain derived from it, BDC2.5, which bears a highly diabetogenic $CD4^+$ T-cell clone specific for a pancreatic β-cell auto-Ags, have been used extensively for the study of peptide-based immunotherapies. Several natural β-cell auto-Ags and their mimotopes, identified through scanning of a synthetic combinatorial peptide library, have been delivered as soluble Ags to these murine models in attempts to induce tolerance. Reduced incidence of T1D or even complete prevention of disease onset have been reported in a number of studies following delivery of these soluble Ags via different routes of administration, including intravenous, oral, intranasal and subcutaneous administration [17]. Major findings of these studies are summarized in the following sections according to the auto-Ag used.

GAD

GAD is the rate-limiting enzyme that converts glutamic acid into γ-aminobutyric acid. Humans express the GAD65 isoform, while mice express the GAD67 isoform [18,19]. GAD is a putative auto-Ag that is targeted during the initiating stages of T1D pathogenesis as suggested by both human epidemiological studies and murine models [7,8,17,20–22]. Intraperitoneal delivery of GAD65-derived peptides to young NOD mice at the time when islet-reactive T cells were first detected protects the recipients from disease development [23–25]. Protection correlates with milder insulitis and reduction of IFN-γ secreting GAD65- and insulin B-chain-specific $CD4^+$ T cells. Expansion of circulating Tregs detected in GAD65 peptide-tolerized mice is thought to contribute to the disease amelioration [26,27]. Intravenous delivery of recombinant GAD65 to NOD mice results in complete protection from the disease [7,28]. Intranasal administration of three GAD65 peptides that were fused to a carrier protein into young NOD mice prevents T1D and inhibits spontaneous Th1 autoimmunity, but fails to establish tolerance when delivered at a later stage of the disease [29]. Additionally, oral administration of diabetogenic auto-Ags is also capable of suppressing the initiation of T1D via induction of T-cell anergy and activation of

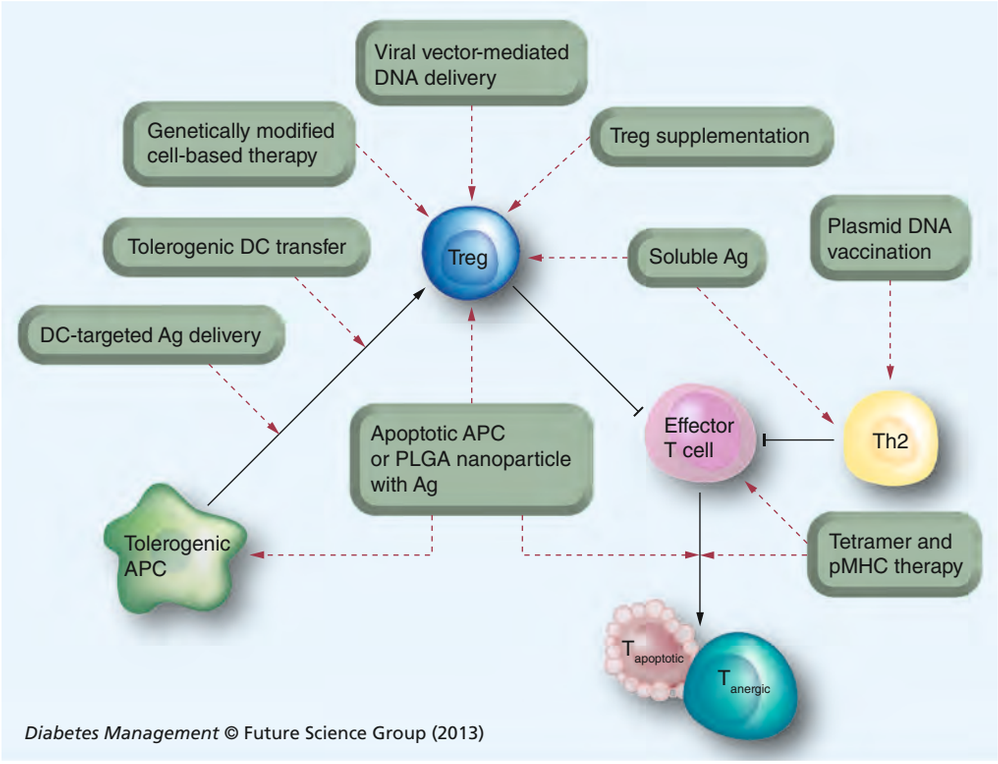

Figure 1. Interventions inducing antigen-specific immune tolerance in Type 1 diabetics.
An overview of the putative mechanisms and cellular targets underlying the diverse antigen-specific immune regulatory strategies that have been employed for the treatment of Type 1 diabetes. Treatments (dashed arrows) targeting a number of these key immune pathways (solid arrows) are being evaluated in clinical settings, including promotion of tolerogenic APCs, inhibition of effector T cells, skewing of T-helper subsets and induction of Tregs through boosting of regulatory pathways. Ag: Antigen; APC: Antigen-presenting cell; DC: Dendritic cell; PLGA: Poly(lactic-*co*-glycolic acid); pMHC: Peptide–MHC complexes; $T_{anergic}$: Anergic T cell; $T_{apoptotic}$: Apoptotic T cell.

Tregs, dependent on the Ag dose [30,31]. Some of these approaches induce GAD-specific Th2 responses, while suppressing Th1 responses. Preclinical and clinical Phase I/II/III trials using recombinant human GAD with or without adjuvants obtained safety evidence for this approach. Only short-term preservation of insulin secretion and fasting C-peptide levels were observed in treated patients [32–38].

■ Insulin & proinsulin

Insulin, as one of the first T1D auto-Ags described, is enzymatically processed from proinsulin [39,40]. Recombinant insulin and its peptides are found to effectively prevent T1D in young NOD mice when delivered via different routes, including intraperitoneal, intravenous, oral and intranasal administration [41–46]. Efficacy is dependent on the combination of peptides used, treatment timing and route of delivery. Certain combinations favor tolerance induction whereas others precipitate the disease. Oral delivery of a soluble form of insulin to a transgenic mouse model (RIP-LCMV) that expresses the viral nucleoprotein of lymphocytic choriomeningitis virus (LCMV) under the control of a rat insulin promoter in β cells, was effective in preventing the development of overt diabetes in more than half of the prophylactically treated mice infected with LCMV as a viral trigger [47]. However, such treatment was ineffective in the prevention of rapid-onset diabetes when using the RIP-GP transgenic mice that express the LCMV glycoprotein. Prevention of diabetes in this model was mediated by Tregs, a mechanism that will be discussed in the following sections in more detail, via bystander suppression, rather than by selective deletion of

insulin-specific effector cells [31]. Intraperitoneal administration of proinsulin fragments to postnatal day 18 NOD mice delays incidence and time of onset of T1D [48]. However, initiation of treatment with proinsulin after development of insulitis accelerates clinical onset of the disease, suggesting that proinsulin may be one of the initiating autoantigenic epitopes in the pathogenesis of T1D, but may become subdominant following epitope spreading [49]. Additionally, prophylactic delivery of a segment of leader sequence in preproinsulin is also capable of inducing tolerance when administered subcutaneously to adult NOD mice in association with the appearance of IL-4- and IL-10-producing Tregs [50]. Clinical trials using insulin prophylactically or therapeutically to treat T1D failed to delay β-cell destruction or delay disease development, but ameliorated insulin-specific T-cell responses [51–56].

■ Hsp60

Hsp-specific T-cell responses are detected in the majority of T1D patients [57,58]. Intranasal delivery of Hsp65 to young NOD mice induced elevated levels of IL-4, -10 and -13, an induction of Ag-specific skewing from a Th1 to Th2 response, in addition to a significant decrease in disease incidence [59–61]. Clinical trials using a mutated form of Hsp60 resulted in transiently stabilized C-peptide production and β-cell function [62,63]. The mechanisms by which Hsp60 affords protection remains elusive.

■ Mimotopes

When natural epitopes from islet β cells are weakly agonistic for autoreactive T cells, mimotopes, peptides that mimic the stimulatory activity of the natural epitopes, can be used to induce tolerance. A recent study demonstrated that subcutaneous delivery of a strong agonistic insulin mimotope for the BDC2.5 T cells to NOD mice at a subimmunogenic dose effectively induced $Foxp3^+$ Tregs, resulting in complete prevention of T1D [64]. Similarly, intravenous administration of a panel of β-cell mimotope peptides to BDC2.5 mice results in protection from T1D [65]. These data suggest that high-affinity peptide analogs of autoimmune epitopes might be useful therapeutic modulators.

■ Challenges to clinical translation

Although soluble peptide immunotherapies are efficacious in animal models of T1D, translation of tolerance therapies to the clinic remains underdeveloped for a number of reasons [7,8,35,48,51,53,54,66–69]. First, human diabetogenic auto-Ags that are critical for T1D progression are less defined; thus, identification of epitopes recognized by pathogenic T cells in humans with diverse genetic backgrounds remains challenging. Second, clonal deletion of autoreactive T cells induced by large doses of soluble Ags is highly Ag specific, and, therefore, may be unable to induce long-term immune tolerance in the background of progressive epitope spreading. Thus, Treg expansion is a preferred alternative as it can regulate autoimmunity independent of Ag specificity; although maintaining sufficient amounts of Treg to counterbalance the increasing frequency of activated autoreactive T cells remains challenging [14,17,70–72].

Tolerance induced by genetic vaccination

Genetic vaccination offers an alternative to re-establishing peripheral tolerance in an Ag-specific manner. Compared with the soluble Ag therapy, gene transfer enables greater flexibility in the manipulation of T-cell responses reflected in the relatively low production cost of plasmid DNA, circumvention of recombinant protein purification and the advantage of targeting the encoded protein to desired cellular compartments by tagging specific signal sequences. Studies using recombinant DNA therapy to manipulate β-cell autoimmunity have largely been restricted to animal models of T1D with limited advances in the clinical setting [73]. Two approaches have been attempted for the delivery of genetic materials: direct injection of plasmid DNA and viral vector-packaged transgenes.

Plasmid DNA

Plasmid DNA vaccination is considered safe as it rarely integrates into the host genome. However, limitations include the low transfection efficiency, the nonspecific cellular targeting, lack of control and sustainment of the expression level of the encoded protein product [74–76]. Delivery of plasmid DNA encoding a number of β-cell auto-Ags, including GAD65, insulin B chain, proinsulin and Hsp60 have been shown to be effective at inhibiting T1D onset/progression in mouse models of T1D [25,77–85].

The efficiency of plasmid DNA-mediated Ag-specific tolerance depends on the context in which the encoded auto-Ag is expressed. For example, prevention of diabetes in NOD mice

that are intramuscularly injected with plasmid DNA encoding a secreted form of GAD65 stabilized by the Fc portion of IgG is markedly more efficient than in the mice receiving an intracellular form of native GAD65. β-cell auto-Ag-encoding DNA is less efficient at suppressing disease progression at advanced stages of T1D [79,86]. Coupling anti-inflammatory cytokines with specific auto-Ags has been explored to enhance the therapeutic outcomes [77,78,86,87]. Coinjection of plasmid DNA encoding GAD65-IgFc and IL-10 enhances accumulation of GAD65-specific Tregs that secrete anti-inflammatory cytokines [77,78]. Inclusion of a CTLA-4 ligand in the auto-Ag plasmid DNA inoculum also enhances β-cell-specific Treg differentiation, resulting in prevention of T1D in young NODs [88]. Regarding the route of plasmid DNA, intramuscular injection of plasmid DNA preferentially induces a Th1 response, which precipitates the disease, whereas intraepidermal injection results in induction of an IL-4-secreting Th2 response [89–91]. However, intramuscular administration of plasmid DNA encoding the insulin B chain has been shown to reduce the incidence of diabetes in more than 50% of treated mice in the RIP-LCMV model through induction of Tregs, which secrete IL-4 and tolerize autoreactive $CD8^+$ T cells in the draining lymph nodes [85]. Similarly, other mucosal routes of delivery, such as the intranasal and oral route, have also been explored to amplify the regulatory activities of T-cell subsets using plasmid DNA [83,84,87,88,92,93].

Translationally, short-term β-cell function and improved glycemic control was sustained for over 12 months in diabetic patients receiving weekly intramuscular injections of plasmid DNA encoding full-length human proinsulin compared with the placebo controls [94]. Importantly, the plasmid DNA vaccine was well tolerated and efficacy correlated with diminished anti-insulin antibody.

■ Viral vector-mediated tolerance induction

DNA delivery using replication-defective viral vectors has greater transduction efficiency than naked plasmid DNA inducing robust expression of encoded auto-Ags in many tissues. However, virus-specific immunity is the main concern. Viral vectors that have been extensively used to treat diseases caused by infectious pathogens or tumors have also been explored in T1D prevention [95]. Recombinant adeno-associated virus (rAAV) vectors devoid of all viral genes have become the preferred gene transfer vehicle [96,97]. Intramuscular delivery of rAAV vectors expressing β-cell auto-Ags, such as proinsulin and GAD65, in conjunction with IL-10 has been shown to prevent diabetes in NOD mice via Treg induction [98–103]. Direct expression of islet β-cell Ags in the pancreas may induce immunoregulation that is not achievable via systemic delivery. For instance, local pancreatic intraductal delivery of the serotype 6 rAAV vector encoding a model Ag, green fluorescent protein (GFP), effectively transduced the majority of β cells, but there was dramatically reduced transgene expression in nonpancreatic tissues despite the fact that the core zone of the islets was not transduced [104]. Significantly, rAAV transduction has no negative impact on β-cell function [104–107].

Cell-based tolerance

Recent studies have highlighted the efficacy of cell-based tolerogenic treatments in preclinical models of T1D. This includes infusion of Tregs or dendritic cells (DCs) with a tolerogenic phenotype.

■ Tregs

Tregs play a central role in protecting against T1D. Autoimmunity is suggested to result from an imbalance or loss of function in Tregs [108]. Direct supplementation of Ag-specific Tregs can confer long-term protection against T1D [109–111]. Compared with polyclonal populations, Ag-specific Tregs display enhanced homing to the pancreatic lymph nodes and pancreas, and increased secretion of regulatory cytokines such as IL-10 [26,112]. Ag-specific Tregs can mediate suppression of both Ag-specific and nonspecific T cells found in the target tissue via bystander suppression [26]. Recent advances in the isolation and *in vitro* expansion of human Tregs have led to initial clinical testing of Tregs for treatment of human disease [113,114]. The therapeutic use of Tregs is complicated by the fact that Tregs may revert back to an effector phenotype *in vivo* [115], suggesting that Tregs are inherently unstable and could possibly contribute to pathogenic immune responses [115]. Additionally, T1D is associated with several immune defects, including genes that impact Treg function such as IL-2. Reduced expression of IL-2 can limit Treg survival and alter the Treg:effector T-cell ratio in favor of promotion of T1D [116]. Additional

studies are needed to address the stability and Ag specificity of Tregs before they can be tested for therapy of T1D.

■ Dendritic cells

DCs possess the ability to regulate the induction of T-cell activation, anergy and regulation. Several studies have shown that transfer of tolerogenic DCs or DCs that have been cultured *in vitro* under tolerogenic-promoting conditions can prevent or protect against the onset of T1D [117–121]. Bone marrow DCs pulsed with apoptotic cells expressing islet cell auto-Ags *in vitro* display a tolerogenic phenotype via downregulation of CD40 and CD86, and reduced production of IL-6 and TNF-α [122]. IL-10-/TGF-β-treated DCs that have been pulsed with insulin can reduce insulin-specific $CD4^+$ T-cell responses in human diabetics [123].

■ Delivery of Ag via apoptotic cells

During natural cell turnover, apoptotic cells release immunosuppressive cytokines and alter surface protein expression [124,125]. This promotes the tolerogenic uptake and processing of cells by macrophages and other phagocytic cells and prevents pathogenic immune response to self-Ags [124,125]. Splenocytes (SPs) pulsed with autoantigenic peptide(s) and fixed with ethylene carbodiimide (ECDI; Ag-ECDI-SP) induce potent and Ag-specific tolerance and have been shown to be an effective therapy in spontaneous transfer and humanized mouse models of T1D [67,126,127]. Ag-ECDI-SP can act directly on activated T cells via MHC-II/T-cell receptor signaling to induce T-cell anergy [128,129]. However, tolerance induction primarily occurs indirectly via modulation of responses in DCs and Tregs after the uptake and representation of Ag-ECDI-SP by host antigen-presenting cells [67,126,127,130]. Protection against the onset of T1D using similar mechanisms can also be seen in NOD mice infused with UVB-treated NIT-1 cells (β-cell line that expresses islet auto-Ags) or the administration of DCs pulsed with islet cell apoptotic bodies expressing β-cell Ags [122,131].

■ Genetically modified cell-based therapies

Recent cell-based therapies have utilized genetic modifications to enhance therapeutic benefit. Mucosal administration of *Lactococcus lactis* that has been genetically modified to secrete both whole proinsulin and IL-10 induces remission of new-onset diabetes in combination with low-dose anti-CD3 [132]. Treatment resulted in the expansion of Tregs in the pancreatic lymph nodes and pancreas. Unlike treatment with anti-CD3 alone, *L. lactis* treatment induced Ag-specific tolerance without altering immune responses to pathogenic foreign Ags. Lentiviral T-cell receptor gene transfer into polyclonal Tregs followed by Ag-specific restimulation *in vitro* can give rise to large numbers of Ag-specific Tregs [133]. Islet-specific Tregs that were retrovirally transduced to ecotopically express Foxp3 reversed hyperglycemia in new-onset diabetics [112]. Ectopic expression of Foxp3 may stabilize Tregs and limit reversion of Tregs to effector cells *in vivo*.

Targeting tolerance pathways *in vivo*

While cell-based approaches show potential for the treatment of T1D, many challenges remain for clinical translation. Tregs can be difficult to isolate, purify and expand, and their instability *in vivo* poses efficacy and safety concerns. It is also costly to produce large numbers of Tregs under good manufacturing practice (GMP) for clinical use. For these reasons, therapies that target components of the tolerogenic pathways *in vivo* or utilize biopolymer platforms, such as biodegradable poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles, to deliver Ag and/or immunomodulatory drugs may be more translatable.

■ Polymer-based delivery of tolerogenic signals

To overcome obstacles posed by cell-based therapy, inert polystyrene beads or US FDA-approved biodegradable PLGA nanoparticles are being explored as substitutes for cellular vehicles. Treatment with nanoparticles containing short antisense primary transcripts of the costimulatory molecules CD40, CD80 and CD86 can downregulate targeted receptors and induce a tolerogenic phenotype in DC populations *in vivo,* and prevent and reverse T1D in the NOD mice [134]. Similar to Ag-ECDI-SP therapy, our preliminary work shows that PLGA nanoparticles that are ECDI coupled with a peptide, protect NOD/scid mice from transfer of T1D with activated BDC2.5 T cells [Miller SD *et al.*, Manuscript in Preparation]. BDC2.5 T cells isolated from treated mice have reduced production of IFN-γ and TNF-α [Miller SD *et al.*, Manuscript in Preparation]. Ongoing studies are determining the exact mechanisms underlying

particle-based tolerance induction. Ag-coupled nanoparticles are taken up by marginal zone macrophages and other phagocytic cells via scavenger receptors, such as MARCO, in a non-inflammatory manner and induce a tolerogenic phenotype in DCs resulting in the induction of Tregs and other tolerogenic pathways to suppress the ongoing autoimmune response [134,135]. PLGA particles are easy to manufacture under GMP conditions. Current studies are testing whether the particles can be modified to target relevant cell types *in vivo* and if encapsulation of inhibitory cytokines, such as IL-10, can enhance their tolerogenic potency.

■ Tetramer & peptide MHC-based therapy

Peptide–MHC complexes (pMHC) have been shown to bind cognate T-cell receptors and modulate T-cell responses. The generation and use of multimeric pMHCs, such as tetramers, are powerful tools used to address T-cell dynamics, distribution and allow phenotypic characterization of Ag-specific T cells [136,137]. The use of pMHC multimers has also been applied for the prediction and treatment of T1D [138,139]. Treatment with pMHC dimers prevented autoimmunity in two transfer models of T1D by inducing IL-10-dependent Tregs [140,141]. More recent studies show IGRP-specific tetramers coupled with saporin toxin can be used therapeutically to target and specifically delete autoreactive T cells *in vivo,* delaying the onset of T1D [142]. Administration of pMHCs coated onto nanoparticles engages T cells in an Ag-specific manner in the absence of costimulatory signals to induce anergy or apoptosis in naive T cells and induces a regulatory phenotype in diabetogenic memory T cells [143]. Treatment with pMHC-nanoparticles containing IGRP Ags prevented T1D in NOD mice and reversed hyperglycemia in new-onset disease by killing auto-Ag-bearing antigen-presenting cells in an IFN-γ-, IDO- and perforin-dependent manner [143].

■ Targeted Ag delivery to DCs *in vivo*

An alternative to administration of Ag-pulsed DCs is selective delivery of diabetogenic Ags to DCs *in vivo* using anti-DEC-205. DEC-205 is a surface receptor mediating endocytosis of captured Ags to late endosomal compartments of tolerogenic DCs [144,145]. Ag delivery via DEC-205 to specialized MHC class II-containing vesicles enhances Ag presentation to T cells promoting tolerance via clonal deletion and Treg induction [145–147]. Treatment with recombinant fusion anti-DEC 205 Ab-containing mimotope sequences for a diabetogenic $CD8^+$ T-cell clone AI4 results in inducing clonal deletion [148]. Administration of a recombinant fusion anti-DEC 205 antibody containing sequences for a pathogenic mimotope peptide 1040–1063 or proinsulin can protect against the onset of hyperglycemia in the BDC2.5 transfer model and spontaneous disease in NOD mice, respectively [149]. Tolerance using the BDC2.5 mimotope and proinsulin was not achieved by clonal deletion but by the induction of Ag-specific Tregs [149].

Conclusion

The greatest advantage of Ag-specific tolerance induction for the treatment of T1D is the specific targeting of pathogenic autoimmune responses without the safety concerns and hazards associated with nonspecific immunomodulators. Treatments with soluble diabetogenic Ags or peptides have shown some potential in preclinical models; however, most have had limited success in human disease. Failure to induce tolerance via soluble Ags may be due to several factors including: suboptimal dosage, route of administration and timing of treatment during the progression of disease; the immune responses to peptides versus intact Ag that may differ due to alternate Ag processing and presentation; and/or different pathogenic contributions of autoantigens in animals models compared with human disease. The identification and use of highly diabetogenic or high-affinity molecular mimotopes important for human pathology is crucial to increasing the efficacy of soluble and antigen-based treatments in clinical trials. Mechanistically, cell-based treatments impact on similar pathways to promote tolerance via transfer or *in vivo* induction of Tregs, modulation of costimulatory molecules such PD-L1 and production of inhibitory cytokines such as IL-10. Both Treg and dendritic cell-based protocols have recently been approved by the steering committee of the NIH TrialNet consortium and are currently being tested in clinical trials [201,202]. Additionally, the targeting of tolerogenic pathways via noncellular platforms such as PLGA nanoparticles or antibodies capable of delivering Ags to target cell populations *in vivo* are being actively explored. Continued development of clinically viable tolerance-based therapies will eventually allow for the safe and effective Ag-specific treatment of autoimmunity.

Future perspective

Tolerogenic strategies that specifically target diabetogenic T cells in the absence of complications of immunosuppression are ideal for the prevention or even the reversal of T1D. Such Ag-based therapies for the treatment of T1D must not only suppress disease-initiating T cells that are already activated, but as importantly, naive autoreactive T cells that may be recruited to further precipitate the disease through epitope spreading against auto-Ags liberated from damaged islet cells. However, the lack of a complete understanding of the underlying immune mechanisms and failure to identify a comprehensive panel of specific auto-Ags and T-cell epitopes preclude a more rational design of effective Ag-specific therapies. Early-phase clinical trials support the use of Ag-coupled cells or biodegradable nanoparticles as a tool for Ag-specific tolerance induction. This approach will hopefully have broad therapeutic utility in the near future. Ag-ECDI-PBL therapy has showed promising results in early clinical trials for multiple sclerosis [150]. Tolerance induction using cell-based therapies employing Ag-ECDI-PBL may be hampered by cost and complexity issues. The use of biodegradable PLGA nanoparticles provides a more stable tolerogenic carrier vehicle that can be readily customized and easily manufactured under GMP conditions. Although effective clinical translation of the Ag-specific treatments developed using animal models remains challenging, preliminary clinical studies have yielded promising results, providing hope for the availability of a more effective tolerogenic therapy for T1D in the near future.

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