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Non-antigenic and antigenic interventions in type 1 diabetes

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Abbreviations: AAb, autoantibodies; CTLA-4, cytotoxic T-lymphocyte antigen 4; GABA, γ-aminobutyric acid; GAD65, 65 kD glutamic acid decarboxylase; IA-2, islet antigen 2; IL-, Interleukin; T1D, type 1 diabetes; T_{reg}, regulatory T cell; ZnT8, zinc transporter 8

Type 1 diabetes (T1D) results from autoimmune destruction of the pancreatic β-cells. Current T1D therapies are exclusively focused on regulating glycemia rather than the underlying immune response. A handful of trials have sought to alter the clinical course of T1D using various broad immune-suppressors, e.g., cyclosporine A and azathioprine.[1–3] The effect on β-cell preservation was significant, however, these therapies were associated with unacceptable side-effects. In contrast, more recent immunomodulators, such as anti-CD3 and antigenic therapies such as DiaPep277, provide a more targeted immunomodulation and have been generally well-tolerated and safe; however, as a monotherapy there appear to be limitations in terms of therapeutic benefit. Therefore, we argue that this new generation of immune-modifying agents will likely work best as part of a combination therapy. This review will summarize current immune-modulating therapies under investigation and discuss how to move the field of immunotherapy in T1D forward.

Introduction

Diabetes mellitus describes the outcome of several metabolic disorders characterized by hyperglycemia, including type 1 diabetes (T1D). In the context of T1D, hyperglycemia typically results from an immunologically driven assault on the β-cells—the insulin-producing cells of the pancreas—leading to insufficient insulin secretion.[4] β-Cell destruction is often rapid in young subjects but more prolonged in adults; this rate, however, is subject to great variability between individuals. The cells that infiltrate the islets and mediate islet loss are mostly T cells[5] and, even though the events leading up to diagnosis of T1D remain obscure, the importance of T cells in T1D pathology is widely accepted. Due to logistical difficulties, human target-tissue is scarce, and many discoveries in T1D have been achieved through studies of the non-obese diabetic (NOD) mouse, a mouse that spontaneously develops immune-mediated diabetes similar to human T1D.[6,7]

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The realization that an islet-specific autoimmune response underlies T1D provides a clear rationale for immunotherapy. The aim of immunotherapy in T1D patients is to specifically silence ongoing autoimmune effector mechanisms, halt β-cell destruction, and thereby preserve endogenous insulin secretion. According to the Diabetes Control and Complications Trial (DCCT),[8] even modest levels of endogenous C-peptide confer long-term health benefits to a patient. Ultimately, immunotherapeutic drugs could delay or even prevent disease in susceptible individuals. Safety considerations are of paramount importance here, as T1D can be effectively managed by exogenous insulin therapy, and the side-effect profile associated with an effective immunotherapy must be favorable enough to justify its use.

Beginning in the early 1980s, attempts to limit or prevent the T1D-associated autoimmune reaction began to emerge. Early immune interventions included subjecting newly diagnosed patients to plasmapheresis or treatment with cyclosporine A. Plasmapheresis often resulted in a reduction in serum autoantibody (AAb) levels, but had limited impact on β-cell preservation.[9] Cyclosporine A treatment, on the other hand, had a dramatic, albeit short-lived, impact on β-cell preservation but was associated with severe side-effects.[1,10] Other, more recent, trials used non-myeloablative bone marrow transfers[11,12] that had significant—but temporary—high-risk benefit. It is now widely accepted that the risk-reward balance of generalized immune suppression for the treatment of T1D is unacceptable. Thus, in order to design successful treatments, it is clear that the field needs to evolve toward development of therapies that more specifically target the diabetogenic immune response.

Non-Antigenic Immune-Modulators

Monoclonal anti-CD3 antibody

T cells play a critical role in the pathogenesis of T1D in rodents and in humans. Monoclonal antibodies specifically targeted against CD3 prevent or reverse T1D in animal models of both spontaneous and virus-induced T1D.[13,14] CD3 is expressed on the surface of T cells as part of the T-cell receptor complex. The mammalian CD3 comprises four chains: CD3γ-, CD3δ-, and two CD3ε chains. Anti-CD3 antibody therapy (OKT3) has been successfully used for preventing transplant rejection; however, this therapy was associated with increased risk of lymphoma

and toxic side-effects resulting from a treatment-induced cytokine storm.[15] More recent work has been aimed at modifying anti-CD3 antibodies to reduce Fc receptor (FcR) binding affinity, thereby limiting cellular activation and cytokine release, while maintaining immunosuppression. Two humanized anti-CD3 monoclonal antibodies (mAb) with reduced FcR binding have been evaluated in human T1D, teplizumab and otelixizumab. Teplizumab is a humanized OKT3 Ab with a mutated C_H2 region; otelixizumab is a humanized chimeric antibody engineered to lack the glycosylation site in the Fc domain.[16,17]

While initial trial results with either teplizumab or otelixizumab were promising, both failed to clearly demonstrate efficacy in phase III trials. In the initial trials, C-peptide levels stabilized or declined more slowly and insulin usage decreased in patients treated with either anti-CD3 mAb for up to 4 y post-treatment.[18-21] Unfortunately, two large subsequent phase III studies did not meet their primary endpoints; however, in the most recent phase III teplizumab trial, data suggested that younger subjects with good metabolic control and higher C-peptide levels, treated soon after diagnosis, appeared to have the greatest therapeutic benefit.[22] The adverse events (AE) profile in the phase III studies was notably mild, enabling the recent initiation of secondary prevention trials (ClinicalTrials.gov NCT01030861). This class of therapeutics holds great potential when employed at the appropriate dose and in the appropriate patient subset.

Cytotoxic T-lymphocyte antigen 4 (CTLA-4) immunoglobulin fusion protein (Abatacept)

CTLA-4 is a homolog of CD28, a T-cell-expressed molecule that binds the B7 complex (CD80/CD86) on antigen presenting cells (APCs) to provide co-stimulation of T cells. CTLA-4 binds the B7 complex with greater affinity, especially CD80, than CD28, blocking CD28-B7 interaction and suppressing T-cell stimulation.[23] CTLA-4 engagement on effector T cells (T_{eff}) induces tryptophan catabolism, thereby downregulating their activity and reducing T-cell proliferation.[24,25] In contrast, blocking CTLA-4 on suppressive T cells, $CD4^+CD25^+$ regulatory T-cells (T_{regs}), promotes organ-specific autoimmunity in mice.[26]

Abatacept is a fusion protein comprising the Fc region of IgG1 and the extracellular domain of CTLA-4. Consequently, abatacept binds the B7 complex on APCs, preventing T-cell co-stimulation. Administration of abatacept (27 infusions over 2 years) resulted in a 9.6-mo delay in C-peptide decrease in newly diagnosed T1D patients compared with placebo-treated subjects.[27] The results suggested that diabetogenic T-cell activation is partly ongoing at the time of clinical diagnosis and that blocking this costimulation pathway could offer temporary benefit. Unfortunately, the effect was short-lived despite prolonged dosing, indicating that the major wave of T-cell activation may occur prior to diagnosis and cannot be fully ameliorated by abatacept treatment post-diagnosis. The drug is now being investigated in secondary prevention (ClinicalTrials.gov NCT01773707) and may hold potential there.

Monoclonal anti-CD20 antibody (rituximab)

While T cells constitute the most prominent players in T1D pathogenesis, B cells can contribute as antigen-presenting cells (APCs) and secrete autoantibodies prior to T-cell-mediated β-cell destruction.[28] Depletion of B cells by administration of the anti-CD20 mAb, rituximab, temporarily and partially preserved β-cell function over a one-year period in T1D patients.[29] In a follow-up study, rituximab administration lead to a decrease in the incidence of asymptomatic EBV reactivation in T1D patients, but an increase in the frequency of asymptomatic viremia caused by polyomaviruses.[30] It is doubtful, in our opinion, that the limited efficacy profile of rituximab in T1D will ever justify the risks associated with a prolonged period of almost complete B-cell depletion.

Anti-IL-1 antibody (anakinra and canakinumab)

Interleukin (IL)-1β receptors are highly expressed on pancreatic β-cells.[31] Additionally, IL-1β can enhance T-cell expansion and differentiation to a pathological phenotype.[32] Hyperglycemia promotes secretion of IL-1β and binding of this proinflammatory cytokine to its receptor can induce β-cell apoptosis;[33,34] however, disruption of this cytokine pathway, using either the human IL-1 receptor antagonist anakinra or the anti-IL-1 mAb canakinumab, has failed to prevent or ameliorate T1D in patients.[35] Except for a small pilot trial with etanercept (anti-TNF), IL-1 remains the only cytokine being investigated in human T1D trials. The hope with anti-cytokine therapy in T1D is to identify a cytokine that controls major downstream effector pathways, as is the case with TNF in rheumatoid arthritis, where this cytokine is at the top of a 'pro-inflammatory cascade'. An excellent case can be made for some other cytokine targets such as IL-6 or IL-21.[36]

Antigenic Therapies

Antigen-specific immunotherapy has the potential to provide a more targeted impact on the underlying pathology that leads to clinical manifestations of T1D without promoting broad suppression and associated side-effects. A number of antigenic targets are currently being evaluated for efficacy in prevention or reversal of T1D in at-risk human populations.

Target antigens in type 1 diabetes

The first indication of β-cell-specific autoimmunity is the detection of autoantibodies (AAb); and, the presence of AAbs in non-diabetic subjects is predictive of T1D development. Anti-insulin AAbs (IAA) appear early during the pre-diabetic phase and are generally among the first AAbs to be detected.[37] This loss of tolerance to insulin may result from reduced insulin expression in the thymus mediated by a variable number of tandem repeats (VNTR) polymorphism in the *INS* promoter.[38-40] Though IAAs are often the first AAb detected, they are not the only AAbs present in most T1D patients. AAbs specific for proinsulin, the 65 kD form of glutamic acid decarboxylase (GAD65), tyrosine phosphatases islet antigen (IA)-2 and IA-2β, or zinc transporter 8 (ZnT8) may also be detected.[41-45] These proteins, plus insulin, represent the major humoral autoantigens in T1D. They all belong to a regulated secretory pathway and, except for GAD65, are localized in the insulin secretory granule or its membrane.[46] Upon exocytosis and dissociation of insulin, the vesicular membrane proteins and the insulin segment B9–B23 are exposed to the extracellular space.[46] Notably, pathogenic T cells recognizing

the B9–B23 segment of the insulin B-chain have been detected in both NOD and T1D patients.[47,48]

GAD is responsible for the biosynthesis of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and, in humans, exists as a 67 kDa (GAD67) and a 65 kDa (GAD65) isoform, encoded by *GAD1* and *GAD2*, respectively.[49] While expression of both *GAD1* and *GAD2* is seen in the brain, only *GAD2* is expressed in human pancreatic β cells;[50] however, its role in the pancreas remains unclear. The increased expression of GAD65 and, consequently, GABA, has been suggested to regulate, or impair, the first phase of glucose-dependent insulin secretion.[51]

IA-2 and IA-2β are transmembrane protein tyrosine phosphatase-like proteins, expressed in the insulin secretory granules of human β-cells, as well as other peptide-secreting endocrine cells and neurons.[52,53] Both are major autoantigens in T1D;[54,55] dendritic cells that are able to process and present soluble IA-2/IA-2β to $CD4^+$ T cells have been identified at the onset of T1D.[56] IA-2 and IA-2β are encoded on different chromosomes; IA-2 is a 979-amino acid protein located on human chromosome 2q35,[54,57] while IA-2β is 986-amino acids long and expressed on human chromosome 7q36.[58]

Zinc transporter 8 (ZnT8) is a transmembrane protein principally transcribed in the pancreatic islets, with highest expression in β cells. It aids in the accumulation of zinc from the cytoplasm into intracellular vesicles and, hence, might be important in providing zinc for the maturation and/or storage of insulin in β cells.[59] Wenzlau et al.[44] found that 60–80% of new-onset T1D patients had anti-ZnT8 AAbs and 26% of individuals with T1D, previously considered AAb-negative, were positive for anti-ZnT8 AAbs. In contrast, <2% of controls and <3% of patients with T2D were positive for anti-ZnT8 AAbs.[44] Additionally, T cells have been identified that are reactive to ZnT8 in human T1D.[60]

Additionally, islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGPR), a member of the glucose-6-phosphatase family that is specifically expressed in the endoplasmic reticulum of the pancreatic β cells, is also a T1D-associated antigen.[61] IGRP is a target of islet-associated, autoreactive $CD8^+$ T cells in both NOD and human T1D.[62]

Animal studies have shown that delivery of antigens via tolerogenic routes[63,64] or using selective tolerizing adjuvants[65] can reestablish immune ignorance of islet proteins. The hypothesis is that self-reactive T-cell species that have escaped thymic selection can be eliminated or functionally silenced after seeing their cognate antigen in the appropriate context. This approach has seen clinical success in conditions such as food allergy,[66] but translation to an autoimmune disease therapy has been difficult. Below we will summarize some of the clinical data with selected antigens in T1D and try to identify options for improvement.

Insulin

Insulin is a major T1D autoantigen. It has been hypothesized that increased insulin production leads to β-cell stress and the presentation of more insulin antigen on MHC class I expressed on β cells, increasing their susceptibility to T-cell killing.[67] Additionally, IAAs are present in the majority of patients at diagnosis and both $CD4^+$[68] and $CD8^+$[69] insulin-specific T cells are detectable in peripheral blood samples from patients. As the only β-cell-restricted islet autoantigen, insulin is an attractive target for antigen-specific treatment.

Subcutaneous (s.c.) insulin

Subcutaneously delivered insulin was evaluated in an early clinical trial that included individuals with islet-specific AAb^+ first-degree relatives.[70] This initial study indicated that s.c. injection of insulin delayed progression to T1D in at-risk individuals; however, it was later reported that many of the subjects who displayed delayed disease onset also expressed protective HLA genotypes.[71] In the Diabetes Prevention Trial (DPT-1), first-degree relatives with islet cell AAbs and a high (>50%) risk of diabetes development over 5 y were treated with s.c. insulin 2′/day and intravenous insulin annually.[72] Though this treatment regimen was safe and well tolerated, it failed to prevent or delay of T1D onset compared with the control group.[72]

Oral insulin

Oral administration of insulin prevents diabetes in animal models of spontaneous[73] and virus-induced T1D;[74] however, the success seen in human trials has been modest, at best. In the above mentioned DPT-1, one treatment arm comprised relatives with an intermediate (26–50%) risk, who were given daily doses of 7.5 mg human insulin crystals in non-enterocoated capsules—in successful animal studies, insulin was orally administered in doses ranging from 1- to 9-mg. Though the study failed to affect diabetes incidence in the study population,[75] post-hoc subgroup analysis demonstrated that diabetes onset was delayed in subjects with high titers of circulating IAA at inclusion.[75] A 13-y follow up of the same group further suggested that β-cell protection was maintained as long as oral insulin-administration was continued;[76] these findings indicated that IAA titers might be an important inclusion criteria for such studies. High IAA titers at treatment initiation may also predict the efficacy of combination treatment with oral insulin and anti-CD3 in NOD.[77] A phase III clinical trial including presence of IAA as an inclusion criterion is currently recruiting (ClinicalTrials.gov NCT00419562). The capacity of oral insulin delivery to reverse disease in newly diagnosed subjects has also been investigated, including the IMDIAB study,[78] the study by the Diabète Insuline Orale group;[79] and the study by McLaren et al.,[80] however, none were efficacious.

Nasal insulin

In 1993, Metzler et al. first demonstrated that inhalation, but not oral administration, of an auto-antigenic peptide could limit autoimmune pathology in a mouse model of multiple sclerosis.[81] Later, in 1996, nasal administration of insulin was evaluated in NOD mice after onset of subclinical diabetes; this therapy reduced islet pathology and diabetes incidence.[82] In contrast, no protection was observed in a double-blind trial, the Diabetes Prediction and Prevention (DIPP) trial in Finland, involving children with a genetic risk for developing T1D who were positive for IAA and daily nasal insulin-administration (1 U insulin/kg bodyweight daily).[83] Another double-blind trial, the Intranasal Insulin Trial I (INIT I) in Australia, in which first-degree relatives of T1D patients, positive for IAA, were treated with nasally administered insulin, demonstrated the safety and tolerability of intranasal insulin.[84] INIT I results indicated that there was some improvement in immune tolerance; these findings are now

being confirmed in a follow-up study, INIT II. This trial is a double-blind, placebo-controlled trial evaluating the impact of nasal administration of 440 IU insulin on T1D development in first-degree relatives of T1D patients, with AAbs against two or more islet antigens. Nasal insulin is administered once daily for seven days and then, once per week for a year. The primary endpoint of INIT II is diagnosis of diabetes within 5 y of treatment initiation.[85] This trial is currently enrolling (ClinicalTrials.gov, NCT00336674).

Plasmid-encoded proinsulin

A recent study evaluated the effectiveness of a proinsulin-encoding plasmid at reducing anti-insulin autoimmunity and delaying β-cell decline in T1D patients. Diabetic subjects over 18 y of age, diagnosed with T1D within 5 y of enrollment, received weekly intramuscular (i.m.) injections of either an engineered DNA plasmid encoding proinsulin (BHT-3021) at 0.3, 1.0, 3.0, or 6.0 mg, or placebo, for 12 weeks.[86] C-peptide was better preserved in the treated group compared with the placebo-treated cohort during dosing, but the effect vanished after drug withdrawal. This was associated with a measurable reduction in the frequency of proinsulin-specific $CD8^+$ T cells but not of T cells reactive to unrelated molecules. The treatment was well-tolerated and safe.[86] These results offer hope that modern antigen-based agents can still provide meaningful benefit in the later stages post-diagnosis.

However, the lack of long-term C-peptide stabilization and the observation that the plasmid may temporarily reduce $CD8^+$ T-cell numbers indicate that the length of dosing may need to be prolonged or the strategy for plasmid-based therapies may need to be further optimized. As discussed by Gottlieb et al.,[87] there are some possible alterations that hold potential for the optimization of plasmid-based therapies. Plasmids encoding multiple islet-specific autoantigens, or multiple plasmids encoding different autoantigens, could offer better tolerance-induction, considering the epitope spreading seen in most cases of T1D. Moreover, steering the immune response to a more T-helper 2 (T_H2)-like phenotype through the addition of genes encoding suppressive cytokines, such as IL-4 or IL-10, inhibiting a T_H1 response, introducing more non-coding GpG hexanucleotide motifs, or beginning therapy after a short period of immune-suppression, *e.g.,* administration of anti-CD3 or anti-CD20, could potentially increase the efficacy of tolerance induction by plasmid-encoded autoantigens.[87]

GAD65

As one of the major autoantigens in T1D, GAD65 has been extensively evaluated as a possible treatment target. Indeed, animal studies have indicated that nasal and intraperitoneal administration of GAD65 and i.m. administration of a GAD65-encoding plasmid DNA delayed or reduced diabetes incidence in NOD mice.[88-90] Inhibition of diabetes progression in vivo following GAD65 administration may be due to the induction of GAD65-specific T_{regs}.[91] The induction of T_{regs} can be enhanced by the incorporation of an adjuvant such as aluminum hydroxide. This adjuvant preferentially induces T_H2 immune responses and can promote expansion of T_{reg} populations. A human recombinant GAD65 has been produced and formulated with aluminum hydroxide (GAD-alum) by Diamyd Medical.[92] A double-blind, dose-finding phase II study using GAD-alum was conducted in 47 patients diagnosed with latent autoimmune diabetes in adults (LADA) in Sweden.[93] Patients were given 2 doses of 4, 20, 100, or 500 μg GAD-alum, or placebo, at weeks 1 and 4. This treatment was well-tolerated. Increased fasting C-peptide levels were seen at 24 weeks post-treatment initiation in only the 20 μg GAD-alum-treated group; this cohort also expressed a higher $CD4^+CD25^+/CD4^+CD25^-$ ratio.[93] Additionally, 5 y after dosing, no severe treatment-related AEs have been reported and fasting C-peptide levels have been preserved in the 4-, 20-, and 100-μg dosing groups.[94] Another phase II trial was conducted with 70 children and adolescents in Sweden (NCT00435981) who received either 20 μL GAD-alum or placebo at the day of enrollment and, again, 4 weeks later. Enrolled subjects had to have a fasting C-peptide above 0.1 nmol/L and detectable anti-GAD65 AAbs (GADA). Patients treated within 6 mo of diagnosis had a significantly lower loss of C-peptide compared with placebo-treated subjects;[95] and, 4 y after administration, fasting C-peptide declined more slowly in these patients.[96] Though this study failed to show a statistically significant difference between treatment groups, treatment was associated with induction of a more favorable immune response, with decreased GAD-specific $CD4^+$ and $CD8^+$ effector T cells and increased GAD65-specific T_{regs}.[97,98] Notably, 4 y after administration, no difference in T_{reg} function was detected between treatment and placebo cohorts.[99]

In 2008, a larger, multi-center phase III study (NCT00723411) was initiated to examine the effect of 2 or 4 doses of 20-μg GAD-alum. Based on the earlier phase II study, fasting C-peptide above 0.1 nmol/L, detectable GADA, and enrollment within 3 mo of T1D diagnosis was set as the inclusion criteria. GAD-alum treatment did not improve β-cell preservation in this study.[100] Similarly, another multi-center phase II study (NCT00529399) initiated by TrialNet, administering 3 injections of GAD-alum to patients diagnosed within 100 d of enrollment, failed to show any difference in the decline of β-cell function between treatment and placebo.[101] GAD-alum treatment in clinical T1D has thus far been disappointing in late-stage development; based on current data, GAD-alum alone is not sufficient for preventing or reversing β-cell decline. In 2009, a secondary prevention study (DiAPREV-IT; NCT01122446) was initiated to investigate the safety and efficacy of GAD-alum treatment as a preventative therapy in at-risk children with multiple islet-specific AAbs. The primary completion date for this study is estimated to be January 2015.

Clinical trial data has failed to confirm earlier findings in the NOD model that indicated that GAD-alum treatment could delay or reduce diabetes incidence. Another, more recent study evaluated the efficacy of GAD-alum in preventing diabetes onset in the NOD and the virally induced RIP-GP model of T1D.[102] In this study, at least three different doses of GAD-alum were investigated, including the 20-μg dose used in human trials. In agreement with previous clinical trials, GAD-alum failed to prevent diabetes in both mouse models.[102]

Heat-shock protein 60 (HSP60, p277)

The stress protein HSP60, thought to be responsible for preventing stress-induced damage to proteins and functioning as a

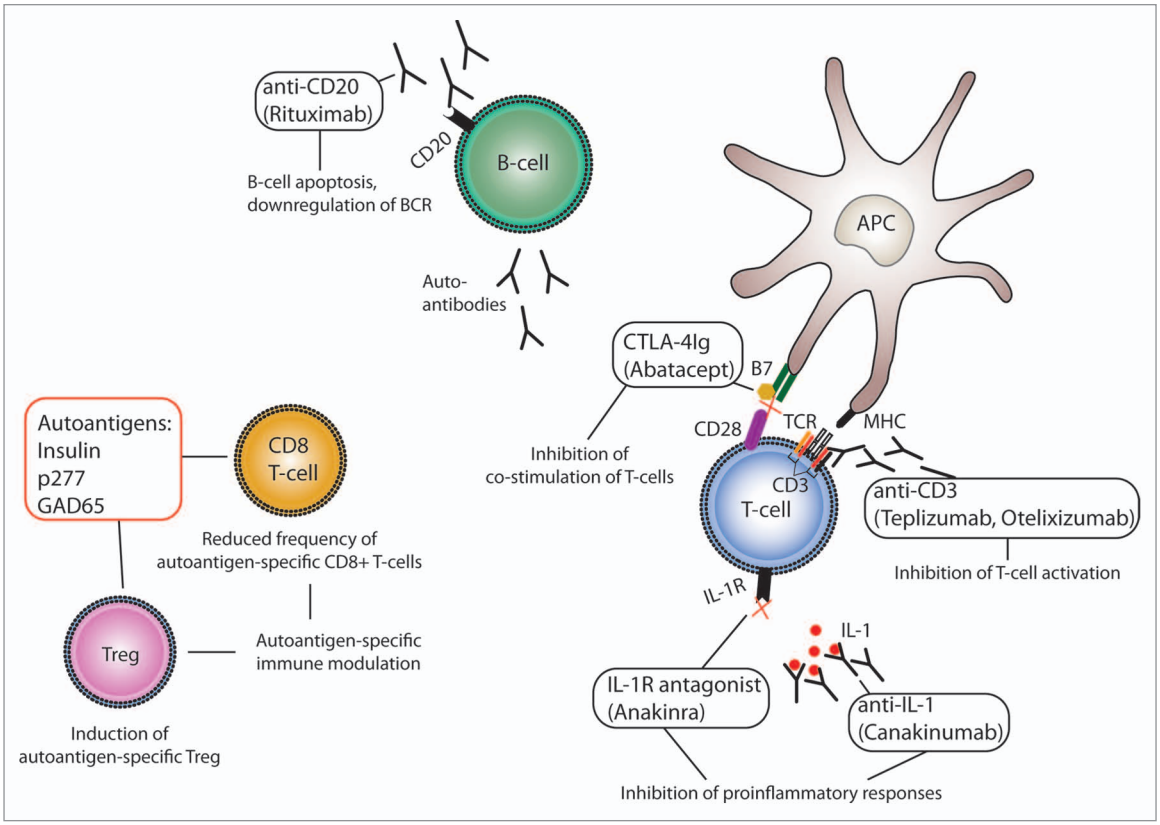

Figure 1. Immune-intervention in T1D. Various therapies of immune-intervention have been investigated in T1D. The fusion protein of cytotoxic T-lymphocyte associated antigen 4 (CTLA-4Ig) inhibits co-stimulation of T cells by competing with CD28 for the binding of B7 on antigen presenting cells (APC), while anti-CD3 (teplizumab and otelixizumab) binding of CD3, as part of the T-cell receptor complex (TCR), leads to the inhibition of T-cell activation. Neutralization of IL-1 by administration of receptor agonist (anakinra) or anti-IL-1 (canakinumab) has the potential to inhibit proinflammatory responses and β-cell apoptosis. Also B cells have a potential role in T1D and anti-CD20 (rituximab) induces downregulation of the B-cell receptor (BCR) as well as B-cell apoptosis. Antigenic therapies are thought to contribute to β-cell preservation by inducing antigen-specific regulatory T cells (T_{reg}) and reducing the frequency of antigen-specific $CD8^+$ T cells.

chaperone protein, has been investigated as a potential autoantigen in T1D. In 1990, HSP60 was shown to be important for the induction of diabetes in the NOD mouse and that transfer of HSP60-reactive T cells could induce diabetes in young NOD mice.[103] Moreover, administration of a peptide (amino acids 437–460) derived from HSP60 (p277) protected NOD mice from both induced and spontaneous diabetes.[104]

DiaPep277 is a stable version of p277 that promotes anti-inflammatory effects and cell adhesion, inhibiting migration and skewing cytokine secretion away from an inflammatory response, through its interaction with Toll-like receptor (TLR) 2. HSP60 acts through TLR4 to promote pro-inflammatory effects; however, DiaPep277 does not impact TLR4 signaling.[105] In a randomized, double-blind, phase II trial, 1 mg DiaPep277 and 40 mg mannitol in vegetable oil, were administered to recent-onset T1D patients at study enrollment and 1- and 6 mo later.[106] Glucagon-stimulated C-peptide production was the primary endpoint of this study, while secondary endpoints were metabolic control and T-cell autoimmunity against HSP60 and p277. At 10 mo, β-cell preservation was seen in the treated group, as shown by better maintained C-peptide concentrations and lower exogenous insulin usage, in comparison to placebo-treated subjects. The DiaPep277-treated individuals also displayed an enhanced T_H2 cytokine profile to HSP60 and p277.[106] Despite these promising results and being safe and well-tolerated in children and adults, several subsequent DiaPep277 clinical trials resulted in modest, if any, effects on β-cell preservation.[65,107,108]

Recently, Andromeda Biotech (TEVA Pharmaceuticals) announced the results from an initial phase III clinical trial, including 457 newly diagnosed T1D patients, aged 16–45 y (NCT00615264). Patients were randomized to either receive s.c. administration of 1 mg DiaPep277 or placebo, once every 3 mo for 2 y. Results from this phase III study are promising, and demonstrated better preservation of C-peptide levels in patients treated with DiaPep277 compared with the placebo arm (http://www.andromeda.com). A confirmatory phase III trial, DIA-AID-2, is currently ongoing and expected to be completed by the end of 2014.

Conclusions

There are a number of therapeutic options for modifying the underlying immune response that mediates β-cell loss and clinical disease (**Fig. 1**). Many of the treatments that held such

promise in mouse models of T1D failed to yield similar results in human populations when administered as monotherapies. Severe side-effects that plagued a number of early immune-interventions are becoming less common as more directed therapeutics are being developed and evaluated. Studies examining the use of insulin as an antigenic therapy in both humans and mice have identified key obstacles that must be overcome before this type of treatment, as well as immune-modifying small molecules, can have therapeutic value in a clinical setting. First, the doses used in human trials have likely been too low to demonstrate sufficient efficacy (~7.5 mg insulin/dose orally in humans vs. 1–9 mg/dose in mice). The optimal dose might greatly depend on the type of insulin administered and route of administration. Notably, orally administered insulin may require enteroprotection as the gastric environment will promote protein degradation, preventing sufficient autoantigen delivery to mucosal tissue and optimal induction of tolerance. Additionally, identifying appropriate inclusion criteria, such as IAA-positivity, could influence treatment outcome.

Second, results from both animal models and human trials have demonstrated that it is most likely easier to prevent diabetes than to stop or reverse its effects after clinical onset. However, T1D prevention studies in humans are complicated. The window of opportunity for treatment is harder to pinpoint in humans and therapies must be demonstrated to be safe in healthy individuals prior to administration to at-risk individuals, who are often children. Even though solid—albeit temporary—effects on β-cell preservation have been shown following immunosuppressive interventions in new-onset and established T1D, the risks associated with long-term immune suppression outweigh these benefits. Antigenic treatment regimens have been safe and well-tolerated, but have had only mild/moderate beneficial effects.

We argue that antigenic therapies may work best in combination with other immune modulators, e.g., one antigenic compound administered with a non-antigenic immunotherapy. Of note, the use of combination therapies is well-supported by an abundance of preclinical data.[16,17,109] Proper dose-finding studies and identification and validation of biomarkers that can identify responders and indicate treatment success early are critical for pushing this therapeutic area forward.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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