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New and future immunomodulatory therapy in type 1 diabetes

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Abstract

Type 1 diabetes is a common autoimmune disease that affects millions of people worldwide and has an incidence that is increasing at a striking rate, especially in young children. It results from the targeted self-destruction of the insulin-secreting β cells of the pancreas and requires lifelong insulin treatment. The effects of chronic hyperglycemia – the result of insulin deficiency – include secondary endorgan complications. Over the past two decades our increased understanding of the pathogenesis of this disease has led to the development of new immunomodulatory treatments. None have yet received regulatory approval, but this report highlights recent progress in this area.

Keywords

type 1 diabetes; immunotherapy; metabolic therapy; biologics

A brief overview of type 1 diabetes

Type 1 diabetes (T1D) (see Glossary) is an autoimmune disease that targets the insulin-secreting β cells of the pancreas, leading to a loss of the ability to regulate blood glucose and other metabolites. The clinical definition of diabetes is based on elevated glucose levels, but does not consider the pathologic process leading to β cell loss. Clinical onset of diabetes is preceded by a prediabetic phase during which effector T cells target β cells. This pathological response is thought to begin with a limited repertoire of antigens and then to expand to additional targets through processes referred to as intra- and inter-epitope spreading. Clinical presentation occurs after significant loss of total β cell mass, which is postulated to be between 70% and 90%. In addition to anatomic loss, dysfunction of the remaining cells could be an important factor at the time of clinical presentation and may explain the ‘honeymoon period’ when insulin secretion from the remaining β cells improves after initial metabolic treatment. During this period, small amounts of exogenous insulin are often sufficient to maintain good metabolic control for a short period of time.

T1D is a complex disease with underlying genetic and environmental components. Class I [1] and Class II [2] MHC haplotypes have been identified that confer both protective and increased risks for disease development. Although some have questioned the significance of the genetic contribution based on discordance among identical twins (as high as 70%), a recent report shows that concordance rates among monozygotic twins approach 100% over their lifetime [3]. In addition to the genetic contribution, environmental factors are also important in disease progression and have been postulated to account for the rising incidence in developed countries – especially in children less than 5 years old [4]. The increased incidence of T1D in youth suggests that children are being exposed to environmental factors

at a very early age or even *in utero*. Currently, large studies such as TEDDY [5] are designed to identify different environmental triggers linked to the disease development.

With this understanding, immunomodulatory therapies with the goal of prevention or treatment based on when therapy is initiated have been tested. Prevention studies strive to re-establish self-tolerance in subjects at risk and to slow progression to full diabetes. Treatment therapies in patients that have developed clinical T1D are targeted to replace, regenerate, and protect the existing β cell mass and insulin secretion. Preservation of residual endogenous insulin secretion at disease onset, in combination with exogenous insulin treatment, may lead to tighter blood glucose control which may result in reduced rates of endorgan complications [6]. Therefore, therapies that preserve some insulin production, even if they are not capable of causing a non-insulin-requiring remission, may have clinical significance.

Recently, the results from Phase II and Phase III clinical trials with new agents have been reported. This review focuses on these recently published results in addition to current trials and the future of metabolic and immunomodulatory therapies for T1D (Table 1).

Types of metabolic and immunomodulatory treatments

The modern era of immunomodulatory therapy in T1D began with trials of the immunosuppressant cyclosporine for treatment of patients with new onset T1D [7,8]. Since then, many different immune-targeted treatments have been developed which either target specific antigens or broad-based immune modulation. These treatments are proposed to work by eliminating or inactivating pathogenic cells and/or by enhancing regulatory cells. Additionally, nutritional studies attempt to modify exposure to different dietary components, which are thought to play a role in disease initiation. The overarching aim of all of these treatments is to restore self-tolerance and prevent the destruction of pancreatic β cells.

Recent trials testing antigen-specific immune modulation

GAD65

GAD65 – a glutamate decarboxylase isoform expressed in β cells – was identified as an autoantigen in T1D more than 20 years ago [9]. The role of an antibody response targeted to this β cell antigen is not completely understood, but is a conserved feature of disease. Preclinical studies have indicated that immunization of NOD (non-obese diabetic) mice with GAD65 before disease onset prevents the development of T1D [10–12] (Box 1). A pilot trial in humans has suggested that immunization with GAD65-alum modulated the disease course in patients with LADA (latent onset diabetes of adults) for up to 5 years [13]. However, two recent clinical trials in humans showed that GAD65-alum did not have a significant effect on the decline in β cell function in patients with recent onset diabetes [14,15]. It is not clear why the murine and human trials were inconsistent, but incongruence in the dose, route, and timing of therapy may have played a crucial role. Multiple different methods of immunization with GAD65 alone or with adjuvant have been effective in preventing onset of T1D in NOD mice [10–12], but the GAD65-alum combination and the subcutaneous route of delivery used in these human trials were not previously tested in preventing disease. Additionally, it is important to note that these clinical trials initiated GAD65 treatment after the onset of T1D in patients, whereas treatment in NOD mice was only effective if started during the prediabetic phase (Table 2).

Box 1**NOD mice as a model for type 1 diabetes**

Since its development in the 1970s the NOD (non-obese diabetic) mouse has been an essential tool for studying the pathophysiology and genetics of T1D. Preclinical studies in NOD mice have often provided the rationale to investigate novel treatments for T1D in humans [77]. Progression of diabetes in this inbred mouse strain is associated with the spontaneous infiltration of leukocytes into pancreatic islets beginning at 4–6 weeks of age and hyperglycemia after 12–14 weeks of age. The disease can be adoptively transferred to immune-deficient mice by splenocytes and even T cells from diabetic animals. Many of the antigens that are involved in human disease, such as insulin, GAD65, and others, are also recognized in this mouse [78]. Unlike disease in humans, diabetes in NOD mice is more frequent in females than in males. Prevention of disease in the NOD model is relatively easy and has been achieved through antigen-specific and non-antigen-specific mechanisms. There are several important differences between this mouse model and clinical T1D which affect translation of the findings in the model. In NOD mice treatment is often initiated before insulinitis, whereas clinical interventions in humans are usually initiated only after a diagnosis of T1D is made. Importantly, at the time of diagnosis, human subjects have already established an autoimmune response and have considerable destruction of β cells mass and progression of the autoimmune response. Nonetheless, the model has been useful for understanding disease mechanisms and for preclinical testing of therapies.

Insulin

Insulin and proinsulin are important antigens in T1D; insulin autoantibodies are generally the first that appear in high risk patients, and preclinical studies have shown that the autoimmune disease is prevented in the absence of native insulin [16]. In NOD mice, both oral [17,18] and intranasal [19] administration of insulin prevented disease development. Trials administering insulin through both oral and intranasal routes have been done in humans. However, in patients, parenteral or oral insulin therapy did not prevent diabetes in high risk individuals [20–22] or slow disease progression in subjects with recent onset T1D [23–25]. Further analysis in these studies [20] found that a subset of patients with elevated insulin autoantibodies at treatment may have received a benefit from oral insulin and a current trial is investigating this further.

A recent trial by Furlanos *et al.* failed to show intranasal insulin decreases β cell destruction in patients with recent onset T1D [25]. However, the trial did show a decrease in the antibody response to insulin with treatment. There is not likely to be clinical significance to the change in antibody titer *per se*, but it may reflect an antigen specific immune modulatory effect. Data from a trial of insulin–DNA plasmid will soon be released. Initial reports suggested an effect of immunization on C-peptide responses, but the trial was not designed to show clinical efficacy [26]. Additionally, Pre-POINT is a current prevention study designed to determine the optimal dose, timing, and administration route of insulin in subjects who have yet to develop autoantibodies but have a high genetic risk for T1D [27].

DiaPep277

DiaPep277 is a synthetic peptide derived from the sequence of human heat shock protein 60 (Hsp60). Initially, Hsp60 was thought to be an autoantigen that was recognized by immune effector cells, but more recent studies have drawn attention to the role of Hsp family proteins in regulating immune responses. Hsp60 functions as a signal to the immune system

by activating anti-inflammatory responses through binding TLR2 and has been postulated to activate CD8⁺ regulatory T cells through the minor Class I MHC molecule HLA-E [28]. Initial studies showed a significant improvement in C-peptide responses in adult male patients treated at baseline, 1 month, 6 months, and 12 months, and followed for a total of 18 months [29–31]. Two pediatric studies failed to show significant improvement in β cell function. However, these studies suggested a preferential effect in subjects expressing HLA-DR4/4 or 3/4 [32,33]. More recently, a Phase III multicenter trial has been reported to have met its primary endpoint of improved C-peptide responses as well as an increased proportion of subjects with a hemoglobin A1c level of 7% or less (<http://www.andromedabio.com/page.php?pageID=69>).

In summary, not all antigen-specific approaches have been successful in preventing or treating T1D, but trials with new agents with novel approaches to antigen delivery (e.g. plasmid DNA), a repeat of the previous oral insulin prevention trial, and report of the final results from the DiaPep277 trial are still pending. Clearly, an antigen-specific approach is most appealing because immunization is generally safe and can be widely available. The mechanisms that are postulated to modulate polyclonal responses from immunization with a single antigen include induction of antigen specific regulatory T cells, secretion of modulatory cytokines, and bystander suppression. However, the dosing or timing of the agent as well as differences in dominant antigens among individuals may be important in optimizing treatment: one antigen may not fit all. The effectiveness of these mechanisms may vary at different disease stages. Therefore, for prevention, at a time when the autoantigenic repertoire is relatively limited, antigen specific therapies are highly desirable.

Nutritional therapies

Omega-3 fatty acids and vitamin D

Trials of both omega-3 fatty acids and vitamin D as therapeutics for T1D were based on pilot clinical trials and observational studies [34,35]. Moreover, the role of vitamin D as an immune modulator, the association between T1D and vitamin D gene polymorphisms, and the relatively frequent deficiency of this vitamin in patients with T1D has prompted interest in a therapeutic trial [36–39]. A recent randomized double-blind controlled pilot study – Nutritional Intervention to Prevent (NIP) Type 1 Diabetes – reported a decline in the level of the stimulated inflammatory mediator IL-1 β in response to treatment with vitamin D and docosahexaenoic acid, an omega-3 fatty acid, but it was not clear whether there were long-term effects on autoimmunity [40]. In addition, a recent randomized double-blind controlled study of 34 subjects found no effect of vitamin D on decline in C-peptide in patients with recent onset diabetes [41].

Cow's milk protein

Dietary exposure to complex proteins, including cow's milk albumin, has been implicated in the development of β cell autoimmunity and increased levels of antibodies to cow's milk protein have been found in children newly diagnosed with T1D [42]. TRIGR (the Trial to Reduce Insulin-Dependent Diabetes Mellitus in the Genetically at Risk) is a prospective, randomized, double-blind study designed to look at the effects of weaning to a highly hydrolyzed casein based formula compared to cow's milk formula in the development of diabetes in high risk individuals [43]. The results from the pilot study of 230 children, which followed subjects for a median of 10 years, showed that high risk patients weaned to a hydrolyzed formula had a decreased development of T1D autoantibodies (adjusted hazard ratio 0.51, $P=0.02$) [44]. This trial did not have the statistical power to determine the effects on the development of T1D. The final TRIGR study will be powered to address this question.

The effects of early infant feeding on the development of autoimmunity raised the interesting possibility that early modulation of the gut microbiome may affect the development of autoimmunity. Recently, evidence to support a significant contribution of the microbiome on T1D in animal models has become available, including a link between innate immune pathways and the acquisition of endogenous flora that may protect from disease [45].

Recent trials testing non-antigen-specific immune modulation

Anti-CD20 mAb

Largely because of the ability of T cells to transfer diabetes in NOD mice, T1D has been considered a T cell-mediated disease and independent of B cells in its later stages. In addition, a case of T1D in a patient with X-linked agammaglobulinemia – in which B cells do not develop – cast doubts on the requirements for B cells in disease progression [46]. However, newer studies have shown that treatment with the B cell depleting anti-CD20 mAb could prevent and reverse T1D in NOD mice, indicating the importance of B cells even in the late stages of disease [47]. In T1D, it is known that B cells function as antigen presenting cells (APCs), activate pathogenic T cells, and produce autoantibodies against pancreatic β cell antigens, but it is not clear when in the disease process these activities are essential [48].

Rituximab is a humanized monoclonal antibody against human CD20 that depletes B lymphocytes. It was originally developed for treatment of B cell lymphomas, but its immunomodulatory effects have led to its use in a variety of autoimmune settings. A recent randomized controlled study showed that treatment of patients with new onset disease reduce the decline in β cell function, as well as levels of glycosylated hemoglobin and insulin use over 1 year [49]. The most significant difference between the rituximab- and placebo-treated cohorts occurred at 3 months post-treatment and after this point their disease courses paralleled each other. Regrettably, the effects of the treatment were not permanent because the significant differences seen at year 1 were no longer apparent at year 2. Deterioration in β cell function was seen as B lymphocytes recovered after drug treatment [50].

These data clearly show that there is a role for B lymphocytes, most likely as APCs, even in the late stages of the disease when patients present with hyperglycemia (Figure 1). Interestingly, further analysis showed patients receiving rituximab treatment had an increased T cell-mediated response to diabetic antigens suggesting that the mechanism was more complex than simple elimination of effector T cells [51].

CTLA4-Ig

T cell activation requires both an antigen-specific signal delivered through the T cell receptor and a co-stimulatory stimulus, which may be delivered through CD28 signaling after binding to CD80 and CD86 on APCs. The importance of the dual signals is greatest for naïve T cells because experienced cells may be activated without co-stimulation [52]. Abatacept (CTLA4-Ig) is a soluble form of the T cell receptor (CTLA-4) that binds CD80 and CD86 and prevents their interaction with CD28, effectively inhibiting co-stimulation and T cell activation.

Based on the understanding that the effectors of auto-immunity have already been activated by the time patients present with T1D, the effects of blockade of co-stimulatory signals on progression of disease were initially unclear. Treatment of NOD mice with human CTLA4-Ig was ineffective in preventing diabetes in NOD mice when it was given after established insulinitis [53] and murine CTLA4-Ig even exacerbated the disease [54]. Nonetheless,

CTLA4-Ig was effective in preventing loss of islet grafts [55] and has been approved for treatment of rheumatoid arthritis, hence the rationale for a clinical trial in T1D.

In a randomized double-blind controlled trial, patients were given either monthly injections of abatacept for 2 years or placebo [56]. After 2 years, the treated group had a 59% higher C-peptide response and decreased glycosylated hemoglobin levels compared with controls while insulin usage was not significantly different. Similar to rituximab, the positive effects of abatacept on β cell destruction were not permanent and after 6 months the drug-treated group paralleled the placebo-treated subjects. It was estimated that treatment with abatacept delayed the decline in C-peptide by 9.6 months on average.

The decreased rate of β cell destruction in the first 6 months of treatment implies that T cell priming and recruitment of new effector cells is still occurring even at the time of clinical presentation (Figure 1). However, despite continued therapy, the effects of the treatment did not persist. The excellent safety profile of abatacept and its ability to prevent naïve T cell activation make it a good candidate for future combination therapies (Box 2).

Box 2

Future treatments

It is becoming clear that the pathogenesis of T1D probably involves a complex balance between T effector and regulatory T cell activity and shifting this balance towards T effectors cells results in development of disease [79]. Therapies that can successfully target and correct this balance should be developed and optimized. In fact, it is proposed that anti-CD3 mAb along with other current immunomodulatory therapies alter this balance in favor of regulatory T cells. Additionally, a current Phase I trial is testing the safety of treatment of patients recently diagnosed with T1D directly with regulatory T cells through a single injection of cells expanded *ex vivo* (see: <http://clinicaltrials.gov/ct2/show/NCT01210664>). Treatments with a single immunomodulatory agent have yet to completely reverse disease in humans; however, combination therapies are starting to be tested. It is logical to think that future therapies will investigate two or more treatments that target different aspects of the pathogenesis of T1D. Treatments that alone were insufficient at reversing disease may be able to establish a more stable remission of disease if administered together [72]. This approach may be useful for improving the efficacy of antigen-specific treatments – for example co-stimulatory modulating treatments in addition to anti-CD3 or anti-CD20 mAbs may be effective. In addition, targeting multiple arms of the immune response (adaptive and innate) may improve the efficacy of each.

Anti-CD3 mAb

FcR binding anti-CD3 treatment selectively targets T cells and has been used to treat allograft rejection in organ transplant patients. Two humanized FcR non-binding anti-CD3 mAbs – teplizumab and otelexizumab – were prepared to reduce cytokine release syndrome and have been used in T1D clinical trials over the past decade [57–59]. Most data from preclinical and clinical studies suggest that the FcR non-binding anti-CD3 mAb induces adaptive regulatory T cells [60,61]. Preclinical studies were compelling and showed that a brief course of drug induced a lasting remission of disease in diabetic NOD mice and achieved immunologic tolerance [60,62]. Clinical trials in humans have shown that short-term treatment with anti-CD3 mAb has a protective effect on β cell function for at least 1 to 2 years in most patients and an effect that may extend up to 4 years or more after treatment [63–66]. Unfortunately, these protective effects eventually diminished and, ultimately, the

disease progressed. Recently, three trials looking at different dosing of anti-CD3 mAb reported their results.

Protégé was a multicenter Phase III international trial that reported the effects of treatment at diagnosis and at 6 months of three different doses of teplizumab [67]. The primary endpoint was a comparison of the proportion of subjects with a glycosylated hemoglobin level of < 6.5% and insulin usage < 0.5 U/kg/day at 1 year. There was no significant statistical difference between the teplizumab-treated and placebo-treated cohorts in this endpoint or in C-peptide responses analyzed with parametric methods. However, the C-peptide data were not normally distributed, and a post-hoc analysis using non-parametric methods showed that, similar to previous reports, treatment with teplizumab did improve insulin secretory responses in patients receiving the ‘full’ dose of the drug ($P=0.046$). The effects of drug treatment on preserving insulin production were most apparent in three predefined subgroups (children aged 8–11 years, the US region and patients randomized 6 weeks after onset). Additionally, in the drug-treated group there was a significant increase in the proportion of subjects who were not taking insulin at the 1-year endpoint, and a significant improvement in the proportion of subjects who used < 0.25 U/kg/day of insulin –the dose administered to non-diabetic relatives of patients in the Diabetes Prevention Trial-1 that did not have significant metabolic effects [21].

The AbATE trial was a multicenter trial conducted in the US by the Immune Tolerance Network that tested whether teplizumab treatment at diagnosis and again at 1 year after diagnosis would improve insulin secretion after 2 years [68]. Subjects were enrolled within 3 months of diagnosis and randomly assigned to a drug treatment or control group. After 2 years, teplizumab-treated patients showed significant improvement in C-peptide responses compared to untreated control subjects. After 2 years, the proportion of control subjects with undetectable C-peptide was more than 8-fold greater than in the drug-treated subjects. In addition, insulin use was significantly reduced in the drug-treated group, which maintained similar or improved hemoglobin A1c levels. However, in both the AbATE and Protégé trials, it is not clear whether there was an effect of the second course of teplizumab treatment on the decline in C-peptide.

The original randomized placebo-controlled study of otelexizumab in new onset T1D showed significant improvement in C-peptide responses over 18 months in subjects who received the drug at diagnosis [65]. The amount of insulin used to maintain hemoglobin A1c levels that were similar to placebo-treated subjects was less in the drug-treated subjects. However, primarily because of the frequent reactivation of Epstein-Barr virus (EBV) in the original trial a Phase III trial (DEFEND-1) was conducted using a much lower dose of the drug to treat individuals with new onset T1D. This trial failed to meet its endpoint, which was a comparison of C-peptide responses at 1 year (see: <http://clinicaltrials.gov/ct2/show/NCT00678886>).

These outcomes illustrate the difficulties in determining drug dosing in T1D trials because a surrogate endpoint is not available. Furthermore, endpoints that indirectly measure insulin production (such as the combination endpoint of hemoglobin A1c and insulin usage in Protégé) may not be useful because of regional differences in treatment of disease and other factors that are unrelated to the effects of the drug on β cell function. For example, insulin use as well as hemoglobin A1c levels were higher at baseline and at later time points in subjects recruited from India compared to the US in the Protégé trial. Importantly, the clinical management of the metabolic disease has improved with the availability of home glucose monitoring, new insulins and insulin delivery systems such as pumps, measurement of hemoglobin A1c, and other factors. Thus, careful consideration of endpoints that reflect the preservation of endogenous insulin production is needed in trial design.

Rapamycin/IL-2

It is thought that in autoimmune diseases the balance between regulatory T cell and effector T cell responses favors effector T cells, and therefore it was proposed that self-tolerance could be re-established if this balance can be restored. Rapamycin, a macrolide, prevents effector T cell proliferation by inhibiting signaling through mTOR [69], whereas low doses of IL-2 have been shown to increase regulatory T cell populations and cause remission of disease in NOD mice [70]. These findings and others suggested that induction of regulatory T cells with IL-2 in combination with selective suppression of effector T cells using rapamycin might slow the disease.

A pilot trial to test the effects of treatment with rapamycin and interleukin-2 (IL-2) was performed in patients with established T1D (4–41 months) and residual insulin production [71]. The combination drug treatment did increase the number of circulating Foxp3⁺ and Helios⁺ regulatory T cells (markers of naturally occurring Tregs). However, all 9 subjects showed a decline in C-peptide responses during drug treatment – 40.5% in 3 months versus 7–14% in historical controls. In most subjects the decline was transient and recovery of pretreatment levels occurred when the drugs were discontinued. This clinical study has raised the question about the dosing of IL-2 that is needed to improve regulatory T cell activity while not enhancing the actions of pathogenic T cells or other immune cells.

Concluding remarks

It has been more than 25 years since cyclosporine was first shown to reduce the decline of insulin production in T1D. Since then there have been many advances in understanding the immunopathogenesis of the disease and we are now seeing the applications of this knowledge to clinical settings. These recent clinical trials, including those with anti-CD20, anti-CD3, CTLA4-Ig, and DiaPep277, have clearly shown how refinements in our knowledge and new reagents can lead to more specific, safer, and even more efficacious treatments than cyclosporine. They have also illustrated how application to the clinical setting requires careful consideration of dosing, timing, and recognition of the differences between human and murine immune responses as well human disease and the preclinical models. Unfortunately, none of the treatments with single agents have achieved stable metabolic remission, and therefore the next step in clinical development will probably involve combinations of agents, based on the understanding of the targeted immune mechanisms to improve the duration and frequency of responses. Combination therapies may lead to more than additive effects because the context in which immune responses are modulated may be important. For example, immunization of an antigen ‘in the setting’ of co-stimulation blockade may induce anergy or a tolerogenic response in antigen-specific T cells, whereas administration of the antigen or co-stimulation blockade alone may not. The recent reports suggest that some tools to achieve this goal may be at hand.

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Glossary

**Antigen-specific
immune
modulation**

the induction of an immune response to a single antigen to treat an autoimmune disease and restore self-tolerance

C-peptide, a peptide secreted in a 1	1 ratio with insulin from pancreatic β cells. It is measured at baseline and in response to an oral glucose load as a measure of β cell function and mass
Effector T cell	a subset of T cells that pathologically attack the pancreatic β cells in T1D
GAD65	an isoform of glutamate decarboxylase found in pancreatic β cells, which catalyzes the formation of the inhibitory neurotransmitter GABA. It is a common autoantigen in T1D
Hemoglobin A1c	is glycosylated hemoglobin formed by the non-enzymatic glycosylation of red blood cells in plasma. Its measurement is used as an estimate of average plasma glucose concentration over the past 3 months
Honeymoon period	the short period of tight glucose control after the precipitating event of T1D before progression to full disease
Insulin	a protein hormone synthesized and secreted by the pancreatic β cells to regulate blood glucose levels. It is a common autoantigen in T1D
Non-antigen-specific therapy	the use of therapeutics that have widespread effects on the immune system to restore self-tolerance
Non-obese diabetic (NOD) mouse	a preclinical model for T1D that has been essential for studying the pathophysiology of this disease
Regulatory T cell	a subset of T cells that function to suppress immune function
Tolerance	the process in which the immune system does not mount an attack against a particular antigen. The immune system normally is self-tolerant, but in autoimmune disease tolerance breaks down
Type 1 diabetes (T1D)	a complex disease with underlying genetic and environmental risk factors that is caused by the autoimmune destruction of the insulin-secreting pancreatic β cells

References

1. Nejentsev S, et al. Localization of type 1 diabetes susceptibility to the MHC class I genes HLA-B and HLA-A. *Nature*. 2007; 450:887–892. [PubMed: 18004301]
2. Erlich H, et al. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk: analysis of the type 1 diabetes genetics consortium families. *Diabetes*. 2008; 57:1084–1092. [PubMed: 18252895]
3. Redondo MJ, et al. Concordance for islet autoimmunity among monozygotic twins. *N Engl J Med*. 2008; 359:2849–2850. [PubMed: 19109586]
4. Harjutsalo V, et al. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. *Lancet*. 2008; 371:1777–1782. [PubMed: 18502302]
5. TEDDY Study Group. The Environmental Determinants of Diabetes in the Young (TEDDY) study: study design. *Pediatr Diabetes*. 2007; 8:286–298. [PubMed: 17850472]
6. Steffes MW, et al. Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care*. 2003; 26:832–836. [PubMed: 12610045]
7. Stiller C, et al. Effects of cyclosporine immunosuppression in insulin-dependent diabetes mellitus of recent onset. *Science*. 1984; 223:1362–1367. [PubMed: 6367043]

8. Bougnères PF, et al. Limited duration of remission of insulin dependency in children with recent overt type I diabetes treated with low-dose cyclosporin. *Diabetes*. 1990; 39:1264–1272. [PubMed: 2210078]
9. Baekkeskov S, et al. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature*. 1990; 347:151–156. [PubMed: 1697648]
10. Tian J, et al. Modulating autoimmune responses to GAD inhibits disease progression and prolongs islet graft survival in diabetes-prone mice. *Nat Med*. 1996; 2:1348–1353. [PubMed: 8946834]
11. Tisch R, et al. Induction of GAD65-specific regulatory T-cells inhibits ongoing autoimmune diabetes in nonobese diabetic mice. *Diabetes*. 1998; 47:894–899. [PubMed: 9604865]
12. Pleau JM, et al. Prevention of autoimmune diabetes in nonobese diabetic female mice by treatment with recombinant glutamic acid decarboxylase (GAD 65). *Clin Immunol Immunopathol*. 1995; 76:90–95. [PubMed: 7606872]
13. Agardh CD, et al. Clinical evidence for the safety of GAD65 immunomodulation in adult-onset autoimmune diabetes. *J Diabetes Complications*. 2005; 19:238–246. [PubMed: 15993359]
14. Werrett DK, et al. Antigen-based therapy with glutamic acid decarboxylase (GAD) vaccine in patients with recent-onset type 1 diabetes: a randomised double-blind trial. *Lancet*. 2011; 378:319–327. [PubMed: 21714999]
15. Ludvigsson J, et al. GAD treatment and insulin secretion in recent-onset type 1 diabetes. *N Engl J Med*. 2008; 359:1909–1920. [PubMed: 18843118]
16. Nakayama M, et al. Prime role for an insulin epitope in the development of type 1 diabetes in NOD mice. *Nature*. 2005; 435:220–223. [PubMed: 15889095]
17. Zhang ZJ, et al. Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. *Proc Natl Acad Sci USA*. 1991; 88:10252–10256. [PubMed: 1946445]
18. Bergerot I, et al. Oral administration of human insulin to NOD mice generates CD4+ T cells that suppress adoptive transfer of diabetes. *J Autoimmun*. 1994; 7:655–663. [PubMed: 7840857]
19. Harrison LC, et al. Aerosol insulin induces regulatory CD8 $\gamma\delta$ T cells that prevent murine insulin-dependent diabetes. *J Exp Med*. 1996; 184:2167–2174. [PubMed: 8976172]
20. Skyler JS, et al. Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial – Type 1. *Diabetes Care*. 2005; 28:1068–1076. [PubMed: 15855569]
21. The Diabetes Prevention Trial – Type 1 Diabetes Study Group. Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N Engl J Med*. 2002; 346:1685–1691. [PubMed: 12037147]
22. Nantö-Salonen K, et al. Nasal insulin to prevent type 1 diabetes in children with HLA genotypes and autoantibodies conferring increased risk of disease: a double-blind, randomised controlled trial. *Lancet*. 2008; 372:1746–1755. [PubMed: 18814906]
23. Pozzilli P, et al. No effect of oral insulin on residual beta-cell function in recent-onset type I diabetes (the IMDIAB VII). IMDIAB Group. *Diabetologia*. 2000; 43:1000–1004. [PubMed: 10990077]
24. Chaillous L, et al. Oral insulin administration and residual beta-cell function in recent-onset type 1 diabetes: a multicentre randomised controlled trial. *Diabète Insuline Orale group*. *Lancet*. 2000; 356:545–549. [PubMed: 10950231]
25. Fourlanos S, et al. Evidence that nasal insulin induces immune tolerance to insulin in adults with autoimmune diabetes. *Diabetes*. 2011; 60:1237–1245. [PubMed: 21307076]
26. Gottlieb, P. Webcast: Interim Results of a Phase I clinical Trial of a DNA Plasmid Vaccine (BHI-3021) for T1D. *DiabetesPro*. 2010. [online], http://professional.diabetes.org/Presentations_Details.aspx?session=3575
27. Achenbach P, et al. Modulating the natural history of type 1 diabetes in children at high genetic risk by mucosal insulin immunization. *Curr Diab Rep*. 2008; 8:87–93. [PubMed: 18445349]
28. Jiang H, et al. HLA-E – restricted regulatory CD8+ T cells are involved in development and control of human autoimmune type 1 diabetes. *J Clin Invest*. 2010; 120:3641–3650. [PubMed: 20877010]
29. Raz I, et al. Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet*. 2001; 358:1749–1753. [PubMed: 11734230]

30. Elias D, et al. DiaPep277 preserves endogenous insulin production by immunomodulation in type 1 diabetes. *Ann N Y Acad Sci.* 2006; 1079:340–344. [PubMed: 17130576]
31. Raz I, et al. Treatment of new-onset type 1 diabetes with peptide DiaPep277[®] is safe and associated with preserved beta-cell function: extension of a randomized, double-blind, phase II trial. *Diabetes Metab Res Rev.* 2007; 23:292–298. [PubMed: 17124720]
32. Lazar L, et al. Heat-shock protein peptide DiaPep277 treatment in children with newly diagnosed type 1 diabetes: a randomised, double-blind phase II study. *Diabetes Metab Res Rev.* 2007; 23:286–291. [PubMed: 17124721]
33. Schloot NC, et al. Effect of heat shock protein peptide DiaPep277 on β -cell function in paediatric and adult patients with recent-onset diabetes mellitus type 1: two prospective, randomized, double-blind phase II trials. *Diabetes Metab Res Rev.* 2007; 23:276–285. [PubMed: 17103487]
34. Stene LC, et al. Use of cod liver oil during the first year of life is associated with lower risk of childhood-onset type 1 diabetes: a large, population-based, case-control study. *Am J Clin Nutr.* 2003; 78:1128–1134. [PubMed: 14668274]
35. Norris JM, et al. Omega-3 polyunsaturated fatty acid intake and islet autoimmunity in children at increased risk for type 1 diabetes. *JAMA.* 2007; 298:1420–1428. [PubMed: 17895458]
36. Pozzilli P, et al. Low levels of 25-hydroxyvitamin D3 and 1, 25-dihydroxyvitamin D3 in patients with newly diagnosed type 1 diabetes. *Horm Metab Res.* 2005; 37:680–683. [PubMed: 16308836]
37. Littorin B, et al. Lower levels of plasma 25-hydroxyvitamin D among young adults at diagnosis of autoimmune type 1 diabetes compared with control subjects: results from the nationwide Diabetes Incidence Study in Sweden (DISS). *Diabetologia.* 2006; 49:2847–2852. [PubMed: 17072585]
38. Driver J, et al. Comparative therapeutic effects of orally administered 1, 25-dihydroxyvitamin D3 and 1 α -hydroxyvitamin D3 on type-1 diabetes in non-obese diabetic mice fed a normal-calcaemic diet. *Clin Exp Immunol.* 2008; 151:76–85. [PubMed: 17983444]
39. Cooper JD, et al. Inherited variation in vitamin D genes is associated with predisposition to autoimmune disease type 1 diabetes. *Diabetes.* 2011; 60:1624–1631. [PubMed: 21441443]
40. Chase, HP. Webcast: Nutritional Intervention to Prevent (NIP) type 1 diabetes, a pilot trial. *DiabetesPro.* 2010. [online], http://professional.diabetes.org/Presentations_Details.aspx?session=3583
41. Bizzarri C, et al. No protective effect of calcitriol on beta-cell function in recent-onset type 1 diabetes. *Diabetes.* 2010; 59:1962–1963.
42. Knip M, et al. Infant feeding and the risk of type 1 diabetes. *Am J Clin Nutr.* 2010; 91:1506–1513.
43. Knip M, et al. Early feeding and risk of type 1 diabetes: experiences from the Trial to Reduce Insulin-dependent diabetes mellitus in the Genetically at Risk (TRIGR). *Am J Clin Nutr.* 2011; 94 (Suppl):1814S–1820S. [PubMed: 21653795]
44. Knip M, et al. Dietary intervention in infancy and later signs of beta-cell autoimmunity. *N Engl J Med.* 2010; 363:1900–1908. [PubMed: 21067382]
45. Wen L, et al. Innate immunity and intestinal microbiota in the development of type 1 diabetes. *Nature.* 2008; 455:1109–1113. [PubMed: 18806780]
46. Martin S, et al. Development of type 1 diabetes despite severe hereditary B-cell deficiency. *N Engl J Med.* 2001; 345:1036–1040. [PubMed: 11586956]
47. Hu CY, et al. Treatment with CD20-specific antibody prevents and reverses autoimmune diabetes in mice. *J Clin Invest.* 2007; 117:3857–3867. [PubMed: 18060033]
48. Wong FS, et al. Investigation of the role of B-cells in type 1 diabetes in the NOD mouse. *Diabetes.* 2004; 53:2581–2587. [PubMed: 15448087]
49. Pescovitz MD, et al. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *N Engl J Med.* 2009; 361:2143–2152. [PubMed: 19940299]
50. Pescovitz, MD. Webcast: Treatment of Type 1 diabetes with Rituxumab — 2 year follow up. *DiabetesPro.* 2010. [online], http://professional.diabetes.org/presentations_details.aspx?session=3463
51. Herold KC, et al. Increased T cell proliferative responses to islet antigens identify clinical responders to anti-CD20 monoclonal antibody (rituximab) therapy in type 1 diabetes. *J Immunol.* 2011; 187:1998–2005. [PubMed: 21775681]

52. Lo DJ, et al. Selective targeting of human alloresponsive CD8⁺ effector memory T cells based on CD2 expression. *Am J Transplant*. 2011; 11:22–33. [PubMed: 21070604]
53. Lenschow DJ, et al. Differential effects of anti-B7-1 and anti-B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse. *J Exp Med*. 1995; 181:1145–1155. [PubMed: 7532678]
54. Salomon B, et al. B7/CD28 costimulation is essential for the homeostasis of the CD4⁺CD25⁺ immunoregulatory T cells that control autoimmune diabetes. *Immunity*. 2000; 12:431–440. [PubMed: 10795741]
55. Londrigan SL, et al. In situ protection against islet allograft rejection by CTLA4Ig transduction. *Transplantation*. 2010; 90:951–957. [PubMed: 20808263]
56. Orban T, et al. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2011; 378:412–419. [PubMed: 21719096]
57. Alegre ML, et al. A non-activating “humanized” anti-CD3 monoclonal antibody retains immunosuppressive properties in vivo. *Transplantation*. 1994; 57:1537–1543. [PubMed: 8009586]
58. Chatenoud L. Immune therapy for type 1 diabetes mellitus –what is unique about anti-CD3 antibodies? *Nat Rev Endocrinol*. 2010; 6:149–157. [PubMed: 20173776]
59. Xu D, et al. In vitro characterization of five humanized OKT3 effector function variant antibodies. *Cell Immunol*. 2000; 200:16–26. [PubMed: 10716879]
60. Chatenoud L, et al. CD3 antibody-induced dominant self tolerance in overtly diabetic NOD mice. *J Immunol*. 1997; 158:2947–2954. [PubMed: 9058834]
61. Belghith M, et al. TGF-beta-dependent mechanisms mediate restoration of self-tolerance induced by antibodies to CD3 in overt autoimmune diabetes. *Nat Med*. 2003; 9:1202–1208. [PubMed: 12937416]
62. Chatenoud L, et al. Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. *Proc Natl Acad Sci USA*. 1994; 91:123–127. [PubMed: 8278351]
63. Herold KC, et al. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med*. 2002; 346:1692–1698. [PubMed: 12037148]
64. Herold KC, et al. Treatment of patients with new onset type 1 diabetes with a single course of anti-CD3 mAb teplizumab preserves insulin production for up to 5 years. *Clin Immunol*. 2009; 132:166–173. [PubMed: 19443276]
65. Keymeulen B, et al. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med*. 2005; 352:2598–2608. [PubMed: 15972866]
66. Keymeulen B, et al. Four-year metabolic outcome of a randomised controlled CD3-antibody trial in recent-onset type 1 diabetic patients depends on their age and baseline residual beta cell mass. *Diabetologia*. 2010; 53:614–623. [PubMed: 20225393]
67. Sherry N, et al. Teplizumab for treatment of type 1 diabetes (Protégé study): 1-year results from a randomised, placebo-controlled trial. *Lancet*. 2011; 378:487–497. [PubMed: 21719095]
68. Gitelman, SE. Webcast: AbATE (Anti-CD3 mAb). *DiabetesPro*. 2011. [online], http://professional.diabetes.org/Presentations_Details.aspx?session=3791
69. Sehgal SN. Rapamune® (RAPA, rapamycin, sirolimus): mechanism of action immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression. *Clin Biochem*. 1998; 31:335–340. [PubMed: 9721431]
70. Grinberg-Bleyer Y, et al. IL-2 reverses established type 1 diabetes in NOD mice by a local effect on pancreatic regulatory T cells. *J Exp Med*. 2010; 207:1871–1878. [PubMed: 20679400]
71. Long SA, et al. Rapamycin plus IL-2 combination therapy in subjects with T1D results in a sustained increase in IL-2 responsiveness and a transient decrease in C-peptide levels. *Diabetes*. 2011; 60 (Suppl 1):A91.
72. Herold KC, Bluestone JA. Type 1 diabetes immunotherapy: is the glass half empty or half full? *Sci Transl Med*. 2011; 3:95fs1.
73. Rabinovitch A, et al. Combination therapy with sirolimus and interleukin-2 prevents spontaneous and recurrent autoimmune diabetes in NOD mice. *Diabetes*. 2002; 51:638–645. [PubMed: 11872661]

74. Elias D, Cohen IR. Peptide therapy for diabetes in NOD mice. *Lancet*. 1994; 343:704–706. [PubMed: 7907681]
75. Elias D, Cohen IR. Treatment of autoimmune diabetes and insulitis in NOD mice with heat shock protein 60 peptide p277. *Diabetes*. 1995; 44:1132–1138. [PubMed: 7657040]
76. Karges W, et al. Immunological aspects of nutritional diabetes prevention in NOD mice: a pilot study for the cow's milk-based IDDM prevention trial. *Diabetes*. 1997; 46:557–564. [PubMed: 9075794]
77. Shoda LKM, et al. A comprehensive review of interventions in the NOD mouse and implications for translation. *Immunity*. 2005; 23:115–126. [PubMed: 16111631]
78. Babad J, et al. T-cell autoantigens in the non-obese diabetic mouse model of autoimmune diabetes. *Immunology*. 2010; 131:459–465. [PubMed: 21039471]
79. Bluestone JA, et al. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature*. 2010; 464:1293–1300. [PubMed: 20432533]

Figure 1. Revised immunologic view of type 1 diabetes

The efficacy of agents such as CTLA4-Ig and anti-CD20 has revised previous concepts about the immunologic mechanisms of the disease. Previously, experimental data suggested that the chronic autoimmune process that leads to β cell destruction was fully matured at the time of clinical presentation and was restricted to T cell-dependent destruction of the cells. The results from these new studies suggest that antigen presentation continues at the time of diagnosis and that there may still be an opportunity to impact the immune progression with agents that can arrest this process. The colored arrows indicate the induction of an autoimmune response to different autoantigens over time. The dashed blue line represents the decrease in β cell mass over time. Treatments that are initiated at clinical presentation (second black arrow) occur after considerable loss in β cell mass. The green dashed line represents the slowed decline in β cell destruction due to an effective treatment. Prevention treatments (first black arrow) are initiated after the presentation of autoantigens, but before a decline in β cell mass. Preclinical studies in NOD mice often initiate treatment even earlier in the disease process before an autoimmune response has developed.

Table 1

Recently completed trials

Trial name	Drug name	Target	Clinicaltrial.gov #	Enrolment	Year/Refs
The Protégé Study – Clinical Trial of MGA031 in Children and Adults With Recent-Onset Type 1 Diabetes Mellitus	Teplizumab	CD3	NCT00385697	International; 516 subjects; 8–35 years old; within 12 weeks of diagnosis	2011 [67]
Autoimmunity-Blocking Antibody for Tolerance in Recently Diagnosed Type 1 Diabetes (AbATE)	Teplizumab	CD3	NCT00129259	US; 81 subjects; 8–35 years old; within 8 weeks of diagnosis	2011 [68]
Trial of Otelixizumab for Adults With Newly Diagnosed Type 1 Diabetes Mellitus (Autoimmune); DEFEND-1	Otelixizumab	CD3	NCT00678886	International; 240 subjects; 12–45 years old; within 90 days of diagnosis	2011 ^a
Effects of Rituximab on the Progression of Type 1 Diabetes in New Onset Subjects	Rituximab	CD20	NCT00279305	International; 87 subjects; 8–45 years old; within 3 months of diagnosis	2009 [49]
Intravenous CTLA4-Ig Treatment in Recent Onset Type 1 Diabetes Mellitus	Abatacept	CD80 and CD86	NCT00505375	US, Canada; 112 subjects; 6–45 years old; within 3 months of diagnosis	2011 [56]
A Phase I Trial of Proleukin and Rapamune in Recent-onset Type 1 Diabetes Mellitus	IL-2 Rapamycin	mTOR, IL-2 receptor	NCT00525889	US; 9 subjects; 18–45 years old; within 3–38 months of diagnosis	2011 [71]
Effects of Recombinant Human Glutamic Acid Decarboxylase (rhGAD65) Formulated in Alum (GAD-alum) on the Progression of Type 1 Diabetes in New Onset Subjects	GAD65-alum	GAD65 antigen-specific treatment	NCT00529399	US; 145 subjects; 3–45 years old; within 3 months of diagnosis	2011 [14]
Efficacy and Safety of Diamyd® in Children and Adolescents With Type 1 Diabetes	GAD65-alum	GAD65 antigen-specific treatment	NCT00435981	Sweden; 70 subjects; 10–18 years old; within 18 months of diagnosis	2008 [15]
Evidence That Nasal Insulin Induces Immune Tolerance to Insulin in Adults With Autoimmune Diabetes	Insulin	Insulin antigen-specific treatment		Australia; 52 subjects; 38–55 years old; within 12 months of diagnosis	2011 [25]
Efficacy Study of DiaPep277 in Newly Diagnosed Type 1 Diabetes Patients (DIA-AID)	DiaPep277	Hsp60	NCT00615264	International; 457 subjects; 16–45 years old; within 3 months of diagnosis	2011 ^b
Nutritional Prevention Pilot Trial for Type 1 Diabetes (MIP)	Cow's milk protein	Cow's milk protein antigen-specific treatment	NCT00570102	Finland; 230 subjects; infants observed until 10 years of age	2010 [44]

^a <http://clinicaltrials.gov/ct2/show/NCT00678886>.

^b <http://clinicaltrials.gov/ct2/show/NCT00615264>.

Table 2

Preclinical versus human studies

Treatment	Preclinical	Human	Comment
Anti-CD3 mAb	Treatment of overtly diabetic NOD mice with anti-CD3 mAb achieved complete and permanent remission of disease mediated through CD4 ⁺ CD25 ⁺ cells [60–62]. However, treatment initiated before insulinitis did not achieve protection or tolerance. Treatment of diabetic NOD mice resulted in a noninvasive and nondestructive form of insulinitis confined to the periphery.	Multiple studies in humans have shown that a single course of treatment decreases β cell destruction over 2 years [63–67]. There is also significant reduction in insulin demand after 4 years and significant improvement in C-peptide after 3 years. Side effects include mild cytokine release and transient EBV reactivation.	The improvement in C-peptide response in humans was not permanent. The mechanisms of action of anti-CD3 mAbs are not well understood.
Anti-CD20 mAb	In a NOD transgenic mice expressing human CD20 on B cells, treatment with human anti-CD20 mAb either transiently depleted B cells and slowed insulinitis and progression to diabetes (if treatment was initiated before insulinitis) or stably reversed disease (if treatment was started after frank hyperglycemia) [47].	A trial in humans transiently depleted B cell counts and significantly improved C-peptide response after 1 year. The treated group had higher C-peptide levels, decreased HbA1c, and decreased insulin usage. The course of disease of the treated and control groups paralleled each other after 3 months, and after 2 years the significant differences seen at 1 year were no longer apparent [49,50].	The exact role of B cells in T1D is still not clear, but they are playing a crucial role late in disease pathogenesis. The positive effects seen in humans are not permanent and the stable remission reported in NOD mice is not achieved.
CTLA4-Ig	Initiation of treatment of NOD mice with human CTLA4-Ig before insulinitis prevented development of disease but had little effect on the severity of insulinitis. Treatment initiated after insulinitis did not alter the normal disease course and did not prevent development of diabetes [53]. Interestingly, treatment of 2- to 4-week-old NOD mice with murine CTLA4-Ig exacerbated the development of diabetes [54].	Monthly injections of CTLA4-Ig over 2 years resulted in increased C-peptide response and decreased HbA1c levels. However, after 6 months the disease course of the treated and control groups paralleled each other. Follow-up studies will determine if significance exists between the treated and control groups after cessation of treatment [56].	The early deviation from disease course in humans suggests that at diagnosis newly activated effector T cells are playing a role in disease progression.
Rapamycin/IL-2	Daily treatment of NOD mice with IL-2 or rapamycin alone was capable of preventing progression to disease, but diabetes developed after treatment ended. However, combination treatment was capable of preventing development of disease with protective effects that extended after treatment ended [73]. A second study showed that initiating 5 days of low dose IL-2 treatment at diabetes onset increased regulatory T cell populations and caused stable remission of disease in some NOD mice [70].	Treatment with rapamycin/IL-2 combination therapy in a pilot study resulted in an increase of circulating Foxp3 ⁺ regulatory T cells, but a significant decrease in C-peptide response, compared with historic controls after 3 months. Some patients showed a transient recovery in C-peptide response after drug administration ended at this time point [71].	In NOD mice the response to treatment was dose dependent and it is possible that differences in dosing may result in unexpected outcomes.
GAD65	In NOD mice a single intraperitoneal injection of GAD65 with Freund's adjuvant [12] and multiple intravenous [10] or intraperitoneal [11] injections of GAD65 alone have been shown to prevent the development of disease and reduce insulinitis.	Two different trials in humans showed multiple subcutaneous injections of GAD65- alum did not have a significant effect on β cell destruction, although it did produce an enhanced immunoglobulin response to GAD65 [14,15].	There are inherent inconsistencies between route of administration, timing and adjuvant used between the preclinical and clinical trials.
Insulin	In NOD mice normal insulin expression is required for the development of T1D [16]. Both oral and nasal routes of administering insulin have been shown to decrease incidence of diabetes and reduce insulinitis in this model [17–19].	Both oral and nasal insulin treatments have failed to show preventative [20–22] or protective [23–25] effects in individuals at high risk for development of, or recently diagnosed with, T1D. However, a subgroup analysis of the Diabetes Prevention Trial-1 did suggest an effect of oral insulin on subjects with high titer of anti-insulin antibodies. Early data from use of an insulin–DNA plasmid suggested an effect on C-peptide [26].	The recent report of tolerance to nasal insulin suggests that the immunologic mechanism may be achievable [25]. In addition, a repeat of the oral insulin trial is ongoing and the trial with the insulin–DNA plasmid has not yet reached its endpoint.

Treatment	Preclinical	Human	Comment
Hsp60	In NOD mice a single inoculation of Hsp60 administered after overt hyperglycemia has been shown to prevent progression of disease and regression of insulinitis [74,75].	Initial results from a recent Phase III trial showed subcutaneous injections of DiaPep277 at 3 month intervals over 2 years resulted in increased baseline C-peptide levels compared to placebo. Phase II studies in men reported improved C-peptide responses after subcutaneous DiaPep277 treatment [29–31]; however, two pediatric studies have failed to show significant improvement in β cell function [32,33].	After treatment was discontinued in the Phase II clinical trials the disease continued to progress. The full results of the recently completed Phase III trial have not yet been published.
Cow's milk protein	A casein-based test diet was effective at preventing T1D in NOD and induced protection to multiple diabetic autoantigens. Treated mice did not develop T1D when co-transferred with splenocytes from diabetic donors [76].	The TRIGR pilot study showed that patients weaned to a hydrolyzed formula compared with a cow's milk based formula had a decreased development of T1D autoantibodies [44].	Although the recently reported pilot trial suggested that early introduction of cow's milk containing formula might affect the early development of autoantibodies, it did not have the statistical power to determine the effects on the development of T1D; the current TRIGR trial will.