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Trials in type 1 diabetes: Antigen-specific therapies

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Abstract

Type 1 diabetes (T1D) results from an aberrant immunological response against the insulin-producing beta cells in the islets of the pancreas. The ideal therapy would restore immune balance in a safe and lasting fashion, stopping the process of beta cell decay. The efficacy of immune suppressive agents such as cyclosporin underscores the notion that T1D can in principle be prevented, albeit at an unacceptable long-term safety risk. Immune modulatory drugs such as monoclonal anti-CD3 antibody, on the other hand, have recently had rather disappointing results in phase 3 trials, possibly due to inadequate dosing or choice of inappropriate endpoints. Therefore, it is argued that striking the right balance between safety and efficacy, together with careful trial design, will be paramount in preventing T1D. Here we outline the concept of antigen-specific tolerization as a strategy to safely induce long-term protection against T1D, focusing on available clinical trial data, key knowledge gaps and potential future directions.

Keywords

Type 1 diabetes; Antigen-specific therapies; Immune intervention; Clinical trials

1. Introduction

The development of dysglycemia in T1D represents the end stage of a period of silent, immune-mediated beta cell decay [1]. Around the time of diagnosis it is estimated that up to 90% of functional beta cell mass is destroyed, although most patients still produce variable amounts of insulin as measured by C-peptide secretion. The natural course of T1D prior to diagnosis remains elusive, but relatively accurate risk predictions can be performed based on genetic screening and detection of islet autoantibodies.

It is well established that effector mechanisms in T1D are primarily T cell-driven, as attested to by the predominance of T cells in the characteristic islet infiltrate after diagnosis [2] and

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KTC and MTvH are both employed by Novo Nordisk.

the ability of certain T cell clones to directly kill beta cells [3]. Replenishing the functional beta cell pool by transplantation or regeneration of insulin-producing cells does not offer a longstanding cure without prevention, since these cells will be recognized and attacked by persisting autoreactive memory T cells [4]. Curing T1D will therefore require a preventative treatment to keep autoreactive T cells in check.

2. Immune suppression versus immune modulation

The outcomes of several trials using immune suppressive or immune modulating agents clearly indicate that interference with the immunological mechanisms of T1D can improve beta cell survival. Immune-mediated interventions in T1D can generally be classified according to their timing relative to clinical diagnosis (Fig. 1). In primary prevention, the aim is to prevent the development of islet autoimmunity, usually defined as the occurrence of islet autoantibodies. In secondary prevention the aim is to prevent healthy autoantibody-positive individuals from progressing to overt dysglycemia. Finally, in intervention studies (sometimes termed ‘tertiary prevention’) the aim is to prevent loss of residual beta cell function after diagnosis, as measured by the primary endpoint of C-peptide secretion, and the secondary endpoints of glycemic indices such as fasting and post-prandial blood glucose, HbA1c or insulin requirement.

Casting any potential therapy within these stages is particularly difficult, as each has its own stringency parameters and ethical considerations (Fig. 1). Thus, primary and secondary prevention may be mechanistically easier to achieve as compared to treatment of advanced disease at diagnosis. From an ethical perspective, however, treatment of healthy individuals demands a zero sum side-effect profile. Animal models roughly support the idea that prevention is indeed easier to achieve earlier, in the pre-clinical stage. Literally hundreds of experimental treatments are known to reduce the incidence of diabetes in the NOD mouse model, yet very few can induce remission after the onset of hyperglycemia [5]. Those that have, e.g. anti-CD3 antibody and bone marrow conditioning, are associated with significant safety risks.

Non-specific immune suppressive agents broadly inhibit the components of the immune system that underlie T1D. Experience with the calcineurin inhibitor cyclosporin A has taught us that preservation of beta cell function can indeed be achieved, with many patients experiencing prolonged periods of insulin independence [6,7]. However, the effect was not sustained upon treatment discontinuation and cyclosporin therapy resulted in accelerated renal dysfunction [8]. Similar risk–benefit considerations apply to the use of autologous hematopoietic stem cell transplantation in T1D [9]. Evidently, these approaches are not suitable for prevention, especially with a target population that is predominantly pediatric.

Thus, the case can be made that chronic non-specific immune suppression has an unacceptable risk–benefit ratio for wide adoption in T1D. Immuno-modulatory treatments, such as biologicals directed against immune receptors, could theoretically curb pathogenic autoimmunity while avoiding many of the risks associated with general immune suppression. Examples from recent trials include CTLA-4Ig [10] (abatacept), anti-CD20 [11] (rituximab) and anti-CD3 [12,13] (teplizumab/otelixizumab). These agents are designed

to block critical signaling pathways in disease-associated leukocyte subsets and/or stimulate the function of regulatory T cells in the hope that a short course will confer long-term tolerance and avoid the need for ongoing treatment. Indeed, it would be difficult to justify chronic co-stimulatory inhibition, B or T cell depletion in T1D. Unfortunately, recent immune modulation trials in recent-onset T1D have shown only short-lasting preservation of C-peptide secretion followed by a decline parallel to placebo.

High hopes were initially held for non-mitogenic anti-CD3 antibodies, based on animal studies indicating that they could effectively reverse diabetes in several T1D models [14,15]. Mechanistically, benefit was achieved via effects on both autoreactive and regulatory T cell compartments and treatment appeared to be reasonably safe. Early clinical trials confirmed these findings and two large phase 3 trials were initiated, which unfortunately failed to meet their primary endpoints [16]. While we will not elaborate on the potential reasons for these failures, we wish to highlight that, as opposed to immune suppressive agents, the efficacy of these more subtle immunomodulatory approaches likely depends on several pivotal parameters, such as dosing, timing, inclusion criteria and defined endpoints. Optimization of these parameters is required lest a beneficial outcome is obscured. On a positive note, the favorable safety profile does not appear to preclude a secondary prevention setting, and TrialNet is currently recruiting for a trial of non-mitogenic anti-CD3 antibody in participants at high risk for T1D (NCT01030861).

In conclusion, the modest preservation of beta cell function achieved with immune modulation would imply that higher doses or longer treatment durations, or combinations with other agents, are required. It is uncertain whether the risk–benefit ratio will remain favorable under such conditions.

3. Antigen-specific tolerance: concepts and advantages

The default immune response to foreign protein antigens is activation. This is unless the antigen is delivered and presented to the immune system in a way that promotes ignorance or tolerance. The mucosal immune system has long been known as a potent inducer of immunological tolerance. In 1911, H. Gideon Wells first described the effect of repeated oral administration of proteins such as hen's egg protein to guinea pigs [17]:

'Guinea-pigs fed upon a certain protein are at first rendered sensitive to this protein. After some time, however, if the feeding is continued they become less sensitive, until they reach an immune or refractory condition so that they do not react to two spaced injections of the fed protein.'

More than one hundred years later, this therapeutic concept is still subject to intense investigation. Indeed, a recent phase 2 clinical trial on oral immunotherapy for egg allergy in children showed remarkable desensitization following daily feeding of high doses (gram range) of egg white [18].

The same concept of antigen-specific oral tolerance induction has proven successful in a range of animal models for autoimmunity, including arthritis [19], multiple sclerosis [20] and type 1 diabetes [21]. Other modes of mucosal (nasal [22,23]) and non-mucosal

(subcutaneous [24]) antigen delivery, alone or in combination with specific adjuvants, were explored and promising data were obtained showing that antigen-specific tolerance induction can prevent and in some instances even revert T1D. Despite the extensive dataset in mouse models, clinical translation has proven extremely difficult in autoimmune diseases. In rheumatoid arthritis, a mixed efficacy record was compiled throughout numerous trials, of which many used different dosages and sources of collagen [25]. A recent phase 3 trial provides further evidence for therapeutic efficacy of oral collagen administration with an advantage over the standard-of-care, methotrexate, and with fewer side effects [26]. In multiple sclerosis, the oral administration of myelin showed initial promise [27], yet a phase 3 trial by the company Autoimmune (Myloral; bovine myelin) was unsuccessful. Here, the lack of proper dose finding studies was criticized, which may have rendered the treatment inefficacious due to suboptimal dosing [28]. Collectively, these studies underscore that whereas antigen-specific therapy is reliably safe, its outcome critically depends on a variety of factors, such as disease stage and dosing, as defined in detail in corresponding animal models. We will further argue below that, within the context of T1D, the path towards clinical translation of antigen-specific therapy for autoimmune disorders can be improved.

The cellular immunology of antigen-specific tolerance is well-characterized in mice and typically involves the generation and expansion of antigen-specific regulatory T cells (Treg) (Fig. 2) [29]. In oral tolerance, the importance of the Peyer's patches as the immunological target should be mentioned, as mice lacking these structures fail to develop tolerance against oral proteins [30]. Gamma delta T cells, the predominant T cell type in the mucosa, are also crucial mediators of oral tolerance [31]. Once generated, antigen-specific Treg then circulate and are capable of regulating inflammatory responses locally where they again see their cognate antigen, e.g. within the pancreatic lymph nodes (PDLN) and islets in T1D (after oral or nasal insulin). The specificity of these cells would theoretically restrict their regulatory potential to the antigen that they recognize. However, a process termed linked suppression, originally discovered in the field of transplantation immunology, is thought to enable the antigen-specific Treg to control responses against antigens that are presented on the same antigen presenting cell (APC). In transplantation, this means that once tolerance is established against antigens in a transplant, linked suppression will occur against any third party antigen that is subsequently co-transplanted with the original tissue [32]. In T1D, the implication is that antigen-specific therapy based on a single islet antigen would ensure that ongoing autoimmune responses against other islet antigens are also regulated. The fact that any antigen other than the one used for tolerization must be presented on the same APC as the tolerizing antigen reduces the likelihood of unwanted suppression of normal immune responses. This concept would explain why mucosal immunization with for instance insulin also regulates T cell responses in the PDLN against other immunodominant islet antigens such as GAD or IGRP. This mechanism is particularly important because significant inter-individual variation exists in the autoreactive T cell repertoire in patients [2] and even in inbred NOD mice [33].

Before proceeding to an overview of clinical trials of antigen-specific therapy in T1D we would like to emphasize the importance of biomarker discovery and validation. A pertinent example is the measurement of autoantibodies in order to predict the development of regulatory responses against an autoantigen. In the NOD mouse, insulin autoantibodies are

considered the most reliable marker of autoimmunity [34]. Bresson and coworkers [35] recently showed that in NOD mice the presence of insulin autoantibodies prior to treatment with oral insulin/anti-CD3 combination predicts a beneficial outcome. Other immune biomarkers such as regulatory cytokine responses to islet antigens [36–38] or autoreactive T cell frequencies [39] could help to better assess the efficacy of tolerization therapy in relation to parameters such as dose and administration route in small proof-of-concept trials. An essential knowledge gap is the extent to which these immune markers vary by individual during the natural course of T1D progression.

4. Antigen-specific therapy trials in T1D

4.1. (Prepro)insulin

Among islet autoantigens in T1D, insulin is the only protein restricted to beta cells (excluding the immune system itself), all other known T1D autoantigens being produced by other non-immune cell types. It is well-known that insulin is a major autoantigen in the NOD model [40,41] and evidence is mounting that insulin-reactive T cells play important roles in T1D. Insulin-specific CD4 [42] and CD8 [43] T cells are found in increased frequencies in the peripheral blood of patients and certain patient-derived PPI-specific CD8 T cells can directly kill beta cells in vitro [3]. Moreover, the insulin gene is second to only MHC class II genes in terms of genetic risk association, and its increased expression in the thymus confers protection [44]. Thus, plenty of reasons exist to assume a cardinal role for insulin as an autoantigen in T1D.

Several trials aimed at tolerizing the immune system against insulin in T1D have been conducted (Table 1). Oral administration of whole insulin has consistently resulted in disease prevention in spontaneous [21] and induced [45] animal models. One conclusion from these studies is that the dose–efficacy curve is markedly bell-shaped [46], i.e. that the dose cannot be too low or too high and therefore needs to be carefully determined. Furthermore, the type of insulin significantly matters, as different optimal doses were found for porcine and human insulin, even though there is very little sequence difference. In addition, denaturing a protein generally abolishes its ability to induce oral tolerance, highlighting the requirement for intact whole protein.

Most mouse studies found an optimal dose within the single digit milligram range, given intra-gastrically twice weekly in a relatively large (~0.5 ml) volume of buffer. The largest human trial that tested oral insulin in a secondary prevention setting was the Diabetes Prevention Trial (DPT-1) trial [47]. Here, a 7.5 mg dose of human insulin crystals was given daily in non-entero-coated capsules with methylcellulose filler. Children who were unable to swallow the capsules were allowed to sprinkle the contents over their food or in juice. The investigators considered extensive breakdown of insulin in the GI tract as beneficial to avoid hypoglycemia, although insulin alone without an adsorption enhancing agent has never been shown to cross the mucosal barrier. The trial showed no overall protective effect, although a subgroup effect was seen in participants with pre-existing high circulating concentrations of insulin autoantibodies. A number of potential explanations exist for the apparent lack of efficacy. First, the dose was arbitrarily chosen and extrapolation from mouse studies would predict an optimal dose that is several hundred fold higher. Second, insulin is particularly

prone to denaturation and degradation, and it can be questioned whether autoantigen would be bioavailable after GI passage. Third, patient inclusion may need to be based on the pre-existence of insulin autoantibodies, as demonstrated in mouse studies [35]. A TrialNet study (NCT00419562) is currently recruiting participants to assess this assumption. Other unsuccessful trials of oral insulin in recent-onset T1D, including the IMDIAB [48], Diabète Insuline Orale group [49] and that by the MacLaren group [50] used similar low doses of insulin, daily dosing and no enteroprotective delivery. Our studies in collaboration with Entelos Inc. showed, at least for tolerization by nasal insulin peptide, that high frequency dosing, used in most mouse studies, is less effective than low frequency dosing [51]. In summary, clinical trials involving oral tolerance in T1D have so far largely disregarded what we think are important lessons from animal models.

An alternative mode of mucosal administration, nasal insulin inhalation, was characterized in the NOD mouse model [22]. Here, it was argued that, in contrast to oral insulin, nasal administration preserves the antigenic structure up until immediate contact with the nasopharyngeal mucosa. The intranasal insulin trial I (INIT I) in humans with islet autoantibodies at risk for T1D demonstrated both safety and biomarker effects [52] and is the basis for the ongoing INIT II with clinical diabetes as the primary outcome measure (reviewed in [53]). However, in the meantime a large trial of daily nasal insulin in islet autoantibody-positive children at much higher risk for T1D than participants in INIT II found no effect on progression to diabetes [54]. A recent trial in new-onset patients also found a lack of effect on C-peptide preservation, yet revealed immunological changes suggestive of tolerance induction [55]. The question therefore remains whether this immunological tolerance signature can be optimized and exploited to confer clinically relevant beta cell preservation. The Pre-POINT trial which is currently enrolling aims to identify optimal timing, disease stage, dose and route of administration by intervening with oral insulin in genetically at-risk children before the appearance of islet autoantibodies [56].

Subcutaneous administration of insulin prior to diagnosis has been explored as a secondary prevention strategy, based on encouraging data in animal models [24] and a small pilot trial [57]. Efficacy was however not substantiated in a large cohort of high-risk individuals in the DPT-1 study group [58]. Alternative subcutaneous vaccination approaches include insulin B chain administration in IFA [37], proinsulin peptide [36] and proinsulin DNA [59]; although some evidence was gathered in support of tolerogenic capacity these remain to be assessed for efficacy in larger trials. So-called altered peptide ligands (APL), i.e. insulin peptides that have been designed to interact with TCRs in a tolerogenic manner, have also been considered, but disease exacerbation with APL was seen in multiple sclerosis and extreme caution is obviously advised [60].

4.2. Glutamic acid decarboxylase (GAD)

More than two decades ago, the T1D-associated beta cell autoantigen, until then known by its molecular weight '64K', was identified as GAD, an enzyme that catalyzes the rate-limiting step in the biosynthesis of the inhibitory neurotransmitter GABA (γ -aminobutyric acid) [61]. At least two isoforms of GAD exist in mammals, with molecular weights of 65 kDa (GAD65) and 67 kDa (GAD67), of which GAD65 is the main immunogenic isoform in

T1D [62]. Autoreactivity against this protein is associated with preclinical and recent-onset T1D [63] and measurement of GAD65 autoantibodies is a sensitive and specific diagnostic tool for islet autoimmunity in modern day clinical practice. Evidence indicates however that GAD65 autoantibodies per se are associated with relatively indolent islet autoimmunity and, in the absence of other islet autoantibodies, have low predictive value [64,65]. GAD65 autoreactivity is also found in a rare neurological condition called Stiff Person Syndrome (SPS), which is characterized by symmetrically increased axial muscle tone and a high prevalence of T1D [66]. Of note, GAD65 autoantibodies in SPS differ from those in T1D in terms of isotype and epitope recognition [67]. GAD is widely distributed in the nervous system but is also found in other tissues, including the pancreatic islets, specifically the beta cells. The NOD mouse also exhibits humoral autoreactivity to endogenous GAD [68], and T cell reactivity to human GAD65 was found to play an essential role in the disease process in this model [69,70]. However, in a subsequent workshop, not all labs could not confirm these T cell results and Jaeckel et al. [71] could not show that transgenic GAD65, unlike proinsulin, prevented diabetes in the NOD mouse. Mouse beta cells express mainly GAD67 while human beta cells express only GAD65. In agreement, various approaches to tolerize the NOD mouse immune system against GAD have proved successful [23,72,73]

The formulation chosen by Diamyd for its clinical trials was subcutaneous GAD65 in aluminum hydroxide (alum), a conventional adjuvant in childhood vaccines. This adjuvant was included in order to steer away from a Th1-dominated cellular immune response in T1D in favor of a Th2 humoral response, and to minimize the antigen dose required [74]. Dose escalation studies performed in adults with T1D revealed an optimal dose of 20 micrograms (given twice, four weeks apart) but not higher or lower, line with the notion that correct dosing is pivotal in antigen-specific tolerance therapy [75]. The effect on insulin dependence in the 20 microgram group was still evident after five years, suggesting long term tolerizing effects [76]. A phase II trial in recently diagnosed children and adolescents with T1D was then conducted and reported delayed loss of C-peptide secretion following a single course of therapy, especially in those with short disease duration [77]. This beneficial effect and associated favorable safety profile was later reported to be preserved after 4 years follow up [78]. Immunological evidence for tolerance induction was gathered, which included the detection of GAD65-specific Treg [79], decreased Ag-specific Th1 responses [80] and T cell inhibitory pathways upon Ag stimulation [81].

A subsequent TrialNet phase 2 trial of GAD-alum in patients with newly diagnosed T1D did not show any clinical benefit. Here, two or three doses of subcutaneous GAD-alum across 4–12 weeks were given to patients within 100 days of diagnosis [82]. The results from a Diamyd phase 3 trial were similarly disappointing, with no significant effects on C-peptide preservation, insulin dose, glycated hemoglobin level, or hypoglycemia rate [83]. A significant problem with these studies has been the lack of proof of concept in mouse models with the GAD-alum drug, which in hindsight makes it impossible to address such issues as dosing and the development of appropriate biomarkers for efficacy. In addition, these stories should caution us about over-interpreting positive phase 2 trials, in which the number of patients is relatively small and the observations might not hold up in larger cohorts, in which greater heterogeneity can be expected. The heterogeneity of T1D natural history, presumably reflecting a range of genetically- and environmentally-determined

pathological mechanisms, is an important reason why we need to develop a ‘personalized’ approach to biomarkers. Nevertheless, the GAD-alum vaccine might still be beneficial for prevention of T1D or could become a component of a combination therapy protocol in recent-onset T1D. An ongoing secondary prevention trial (DIAPREV-IT; NCT01122446) may provide more information.

4.3. 60 kDa heat-shock protein (HSP60, p277)

The role of HSP60 as a putative autoantigen in the NOD mouse was first reported by Elias and coworkers [84]. Later, a specific HSP60-derived peptide (p277) was identified that had the ability to confer protection in mice [85,86] and to induce disease in non-susceptible strains after vaccination [87]. Some support exists for an antigenic role for hsp60 in patients with T1D [88], yet the available data indicate a less immunodominant role in comparison to insulin and possibly GAD65.

The compound tested in clinical trials (DiaPep277, TEVA Pharmaceuticals) contains two amino acid substitutions in the native p277 sequence to stabilize the peptide, but does not function as an APL. A pilot trial in 2001 tested the effect of three subcutaneous 1 mg p277 injections in a 10% lipid solution, at entry, 1 month, and 6 months in recently diagnosed T1D patients [89]. Positive outcomes were recorded pertaining to glucagon-stimulated (GST) C-peptide preservation and exogenous insulin requirements. Interestingly, a temporary increase of glucagon-stimulated C-peptide in the treatment group was observed after each P277 injection. These data generated support for the idea that a highly safe antigen-specific treatment could reverse the natural course of T1D even after clinical diagnosis. The mechanism of action was proposed to involve altered (Th2-skewed) T cell reactivity to hsp60 and p277, and also direct binding to TLR2 on Treg, enhancing their regulatory potential [90]. A number of other small trials were conducted, which all showed excellent safety, yet were not powered to provide conclusive evidence for efficacy [91–94]. In the trial by Huurman et al. [38] the clinical response to p277 was associated with proliferation of T cells to p277, which might serve as a biomarker.

TEVA Pharmaceuticals has now completed a phase 3 trial, in which preservation of C-peptide secretion to glucagon (but not to a mixed meal tolerance test) showed reduction in the frequency of hypoglycemia and in insulin usage (<http://www.andromedabio.com/news.php>). It is interesting to note that the dose that was used (1 mg s.c. at monthly intervals in oil-based adjuvant) was associated with reduction of hypoglycemia frequency but preservation of C-peptide secretion was optimal at higher doses, as in Huurman et al. (lowest decrease of C-peptide at 2.5 mg) [91]. A further phase 3 trial has recently completed recruitment. Taken together, these data offer substantial grounds for the hypothesis that antigen-specific therapies are safe and powerful enough to tackle even the advanced stages of disease. The immune biomarkers need to be further developed but preliminary data suggest that efficacy at least in part correlates with an altered immune response against the immunizing antigen.

5. Conclusions

There is now reason to be cautiously optimistic that immune intervention can alter the course of beta cell decline in T1D. However, we have yet to find a therapy that strikes the right balance between safety and efficacy. The risks associated with chronic immune suppression clearly outweigh the benefits and these drugs have failed to stably restore immune tolerance. Immune modulatory agents such as anti-CD3 antibody have so far not fulfilled their potential in large clinical trials and other biologicals have provided only temporary stabilization of C-peptide secretion. We have argued here that antigen-specific therapies may represent a more suitable approach in T1D, in particular given the excellent safety record compiled across various clinical studies.

Has the clinical potential of antigen-specific therapy for T1D been unequivocally demonstrated in the clinic? Certainly not. The trial data with p277 in recent-onset T1D actually represents the first and only available phase 3 evidence of a tolerizing effect after antigen-specific therapy. The efficacy record of oral and nasal antigen delivery in animal models has thus far failed to move forward from bench to bedside. Of note, many antigen-specific therapies tend only to work prior to onset in pre-clinical models, whereas most clinical testing occurs in a recent-onset setting. Quite clearly, ethical considerations and the shorter trial duration are at play but the choice to treat very advanced disease also sets the bar for clinical efficacy very high. Here, we believe that combination therapies may be the answer. One important advantage could be that the complementary action of two drugs may allow dose reductions and thereby the risk for side-effects. Additionally, it is easy to envision how two drugs with unrelated modes of action could impact the immune system more broadly, confer better protection and perhaps even induce remission. There is pre-clinical evidence to support this line of thought, for instance showing improved efficacy after combining nasal insulin and anti-CD3 [95]. Promising new formulation approaches, such as antigen-coated microparticles [96], peptide-MHC-based nanovaccines [97] or antigen-linked autologous cell transfer [98], could serve as alternatives to improve the efficacy of antigen-specific tolerization.

Finally, it can be argued that better informed decisions are required on the important variables of antigen dose-formulation and frequency, and disease stage, of administration. Despite the shortfalls of animal models, they are undeniably still the best available means of evaluating the aforementioned parameters. Finally, to better position and assess the outcome of antigen-specific interventions, we require suitable immune biomarkers to identify the most appropriate target population and correlate immune function with clinical benefit. Failure to consider the importance of these variables in past trials is a lesson for the future that may eventually allow the translation of antigen-specific therapies from mouse to man.

References

1. van Belle TL, Coppeters KT, von Herrath MG. Type 1 diabetes: etiology, immunology, and therapeutic strategies. *Physiol Rev.* 2011; 91:79–118. [PubMed: 21248163]
2. Coppeters KT, Dotta F, Amirian N, Campbell PD, Kay TW, Atkinson MA, Roep BO, von Herrath MG. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. *J Exp Med.* 2012; 209:51–60. [PubMed: 22213807]

3. Skowera A, Ellis RJ, Varela-Calvino R, Arif S, Huang GC, Van-Krinks C, Zaremba A, Rackham C, Allen JS, Tree TI, Zhao M, Dayan CM, Sewell AK, Unger WW, Drijfhout JW, Ossendorp F, Roep BO, Peakman M. CTLs are targeted to kill beta cells in patients with type 1 diabetes through recognition of a glucose-regulated preproinsulin epitope. *J Clin Invest.* 2008; 118:3390–3402. [PubMed: 18802479]
4. Tyden G, Reinholt FP, Sundkvist G, Bolinder J. Recurrence of autoimmune diabetes mellitus in recipients of cadaveric pancreatic grafts. *N Engl J Med.* 1996; 335:860–863. [PubMed: 8778604]
5. Shoda LK, Young DL, Ramanujan S, Whiting CC, Atkinson MA, Bluestone JA, Eisenbarth GS, Mathis D, Rossini AA, Campbell SE, Kahn R, Kreuwel HT. A comprehensive review of interventions in the NOD mouse and implications for translation. *Immunity.* 2005; 23:115–126. [PubMed: 16111631]
6. Feutren G, Papoz L, Assan R, Vialettes B, Karsenty G, Vexiau P, Du RH, Rodier M, Sirmaj J, Lallemand A. Cyclosporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset. Results of a multicentre double-blind trial. *Lancet.* 1986; 2:119–124. [PubMed: 2873396]
7. Cyclosporin-induced remission of IDDM after early intervention. Association of 1 yr of cyclosporin treatment with enhanced insulin secretion. The Canadian–European Randomized Control Trial Group. *Diabetes.* 1988; 37:1574–1582. [PubMed: 2903105]
8. Parving HH, Tarnow L, Nielsen FS, Rossing P, Mandrup-Poulsen T, Osterby R, Nerup J. Cyclosporine nephrotoxicity in type 1 diabetic patients. A 7-year follow-up study. *Diabetes Care.* 1999; 22:478–483. [PubMed: 10097932]
9. Voltarelli JC, Couri CE, Stracieri AB, Oliveira MC, Moraes DA, Pieroni F, Coutinho M, Malmegrim KC, Foss-Freitas MC, Simoes BP, Foss MC, Squiers E, Burt RK. Autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA.* 2007; 297:1568–1576. [PubMed: 17426276]
10. Orban T, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Gottlieb PA, Greenbaum CJ, Marks JB, Monzavi R, Moran A, Raskin P, Rodriguez H, Russell WE, Schatz D, Wherrett D, Wilson DM, Krischer JP, Skyler JS. 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]
11. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, Becker DJ, Gitelman SE, Goland R, Gottlieb PA, Marks JB, McGee PF, Moran AM, Raskin P, Rodriguez H, Schatz DA, Wherrett D, Wilson DM, Lachin JM, Skyler JS. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *N Engl J Med.* 2009; 361:2143–2152. [PubMed: 19940299]
12. Herold KC, Hagopian W, Auger JA, Poumian-Ruiz E, Taylor L, Donaldson D, Gitelman SE, Harlan DM, Xu D, Zivin RA, Bluestone JA. Anti-CD3 monoclonal antibody in new-onset type 1 diabetes mellitus. *N Engl J Med.* 2002; 346:1692–1698. [PubMed: 12037148]
13. Keymeulen B, Vandemeulebroucke E, Ziegler AG, Mathieu C, Kaufman L, Hale G, Gorus F, Goldman M, Walter M, Candon S, Schandene L, Crenier L, De BC, Seigneurin JM, De PP, Pierard D, Weets I, Rebello P, Bird P, Berrie E, Frewin M, Waldmann H, Bach JF, Pipeleers D, Chatenoud L. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med.* 2005; 352:2598–2608. [PubMed: 15972866]
14. Chatenoud L, Thervet E, Primo J, Bach JF. Anti-CD3 antibody induces long-term remission of overt autoimmunity in nonobese diabetic mice. *Proc Natl Acad Sci U S A.* 1994; 91:123–127. [PubMed: 8278351]
15. von Herrath MG, Coon B, Wolfe T, Chatenoud L. Nonmitogenic CD3 antibody reverses virally induced (rat insulin promoter-lymphocytic choriomeningitis virus) autoimmune diabetes without impeding viral clearance. *J Immunol.* 2002; 168:933–941. [PubMed: 11777992]
16. Sherry N, Hagopian W, Ludvigsson J, Jain SM, Wahlen J, Ferry RJ Jr, Bode B, Aronoff S, Holland C, Carlin D, King KL, Wilder RL, Pillemer S, Bonvini E, Johnson S, Stein KE, Koenig S, Herold KC, Daifotis AG. Teplizumab for treatment of type 1 diabetes (Protege study): 1-year results from a randomised, placebo-controlled trial. *Lancet.* 2011; 378:487–497. [PubMed: 21719095]
17. Wells HG. Studies on the Chemistry of Anaphylaxis (III), Experiments with Isolated Proteins, Especially Those of the Hen's Egg. 1911

18. Burks AW, Jones SM, Wood RA, Fleischer DM, Sicherer SH, Lindblad RW, Stablein D, Henning AK, Vickery BP, Liu AH, Scurlock AM, Shreffler WG, Plaut M, Sampson HA. Oral immunotherapy for treatment of egg allergy in children. *N Engl J Med.* 2012; 367:233–243. [PubMed: 22808958]
19. Nagler-Anderson C, Bober LA, Robinson ME, Siskind GW, Thorbecke GJ. Suppression of type II collagen-induced arthritis by intragastric administration of soluble type II collagen. *Proc Natl Acad Sci U S A.* 1986; 83:7443–7446. [PubMed: 3463976]
20. Higgins PJ, Weiner HL. Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein and its fragments. *J Immunol.* 1988; 140:440–445. [PubMed: 2447178]
21. Zhang ZJ, Davidson L, Eisenbarth G, Weiner HL. Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. *Proc Natl Acad Sci U S A.* 1991; 88:10252–10256. [PubMed: 1946445]
22. Harrison LC, Dempsey-Collier M, Kramer DR, Takahashi K. Aerosol insulin induces regulatory CD8 gamma delta T cells that prevent murine insulin-dependent diabetes. *J Exp Med.* 1996; 184:2167–2174. [PubMed: 8976172]
23. Tian J, Atkinson MA, Clare-Salzler M, Herschenfeld A, Forsthuber T, Lehmann PV, Kaufman DL. Nasal administration of glutamate decarboxylase (GAD65) peptides induces Th2 responses and prevents murine insulin-dependent diabetes. *J Exp Med.* 1996; 183:1561–1567. [PubMed: 8666914]
24. Atkinson MA, Maclaren NK, Luchetta R. Insulinitis and diabetes in NOD mice reduced by prophylactic insulin therapy. *Diabetes.* 1990; 39:933–937. [PubMed: 2197139]
25. Macfarlane GJ, El-Metwally A, De S, Ernst VE, Dowds GL, Moots RJ. Evidence for the efficacy of complementary and alternative medicines in the management of rheumatoid arthritis: a systematic review. *Rheumatology (Oxford).* 2011; 50:1672–1683. [PubMed: 21652584]
26. Wei W, Zhang LL, Xu JH, Xiao F, Bao CD, Ni LQ, Li XF, Wu YQ, Sun LY, Zhang RH, Sun BL, Xu SQ, Liu S, Zhang W, Shen J, Liu HX, Wang RC. A multicenter, double-blind, randomized, controlled phase III clinical trial of chicken type II collagen in rheumatoid arthritis. *Arthritis Res Ther.* 2009; 11:R180. [PubMed: 19951408]
27. Weiner HL, Mackin GA, Matsui M, Orav EJ, Khoury SJ, Dawson DM, Hafler DA. Double-blind pilot trial of oral tolerization with myelin antigens in multiple sclerosis. *Science.* 1993; 259:1321–1324. [PubMed: 7680493]
28. Quinn S. Human trials scientists, investors, and patients in the quest for a cure. 2001
29. Peakman M, von HM. Antigen-specific immunotherapy for type 1 diabetes: maximizing the potential. *Diabetes.* 2010; 59:2087–2093. [PubMed: 20805382]
30. Fujihashi K, Dohi T, Rennert PD, Yamamoto M, Koga T, Kiyono H, McGhee JR. Peyer's patches are required for oral tolerance to proteins. *Proc Natl Acad Sci U S A.* 2001; 98:3310–3315. [PubMed: 11248075]
31. Locke NR, Stankovic S, Funda DP, Harrison LC. TCR gamma delta intraepithelial lymphocytes are required for self-tolerance. *J Immunol.* 2006; 176:6553–6559. [PubMed: 16709812]
32. Davies JD, Leong LY, Mellor A, Cobbold SP, Waldmann H. T cell suppression in transplantation tolerance through linked recognition. *J Immunol.* 1996; 156:3602–3607. [PubMed: 8621893]
33. Coppeters KT, Amirian N, von Herrath MG. Incidental CD8 T cell reactivity against caspase-cleaved apoptotic self-antigens from ubiquitously expressed proteins in islets from prediabetic human leucocyte antigen-A2 transgenic non-obese diabetic mice. *Clin Exp Immunol.* 2011; 165:155–162. [PubMed: 21605113]
34. Bonifacio E, Atkinson M, Eisenbarth G, Serreze D, Kay TW, Lee-Chan E, Singh B. International workshop on lessons from animal models for human type 1 diabetes: identification of insulin but not glutamic acid decarboxylase or IA-2 as specific autoantigens of humoral autoimmunity in nonobese diabetic mice. *Diabetes.* 2001; 50:2451–2458. [PubMed: 11679421]
35. Mamchak AA, Manenkova Y, Leconet W, Zheng Y, Chan JR, Stokes CL, Shoda LK, von HM, Bresson D. Preexisting autoantibodies predict efficacy of oral insulin to cure autoimmune diabetes in combination with anti-CD3. *Diabetes.* 2012; 61:1490–1499. [PubMed: 22362174]

36. Thrower SL, James L, Hall W, Green KM, Arif S, Allen JS, Van-Krinks C, Lozanoska-Ochser B, Marquesini L, Brown S, Wong FS, Dayan CM, Peakman M. Proinsulin peptide immunotherapy in type 1 diabetes: report of a first-in-man Phase I safety study. *Clin Exp Immunol.* 2009; 155:156–165. [PubMed: 19040615]
37. Orban T, Farkas K, Jalahej H, Kis J, Treszl A, Falk B, Reijonen H, Wolfsdorf J, Ricker A, Matthews JB, Tchao N, Sayre P, Bianchine P. Autoantigen-specific regulatory T cells induced in patients with type 1 diabetes mellitus by insulin B-chain immunotherapy. *J Autoimmun.* 2010; 34:408–415. [PubMed: 19931408]
38. Huurman VA, van der Meide PE, Duinkerken G, Willemen S, Cohen IR, Elias D, Roep BO. Immunological efficacy of heat shock protein 60 peptide DiaPep277 therapy in clinical type I diabetes. *Clin Exp Immunol.* 2008; 152:488–497. [PubMed: 18422727]
39. Velthuis JH, Unger WW, Abreu JR, Duinkerken G, Franken K, Peakman M, Bakker AH, Reker-Hadrup S, Keymeulen B, Drijfhout JW, Schumacher TN, Roep BO. Simultaneous detection of circulating autoreactive CD8+ T-cells specific for different islet cell-associated epitopes using combinatorial MHC multimers. *Diabetes.* 2010; 59:1721–1730. [PubMed: 20357361]
40. Nakayama M, Abiru N, Moriyama H, Babaya N, Liu E, Miao D, Yu L, Wegmann DR, Hutton JC, Elliott JF, Eisenbarth GS. Prime role for an insulin epitope in the development of type 1 diabetes in NOD mice. *Nature.* 2005; 435:220–223. [PubMed: 15889095]
41. Narendran P, Mannering SI, Harrison LC. Proinsulin-a pathogenic autoantigen in type 1 diabetes. *Autoimmun Rev.* 2003; 2:204–210. [PubMed: 12848947]
42. Oling V, Marttila J, Ilonen J, Kwok WW, Nepom G, Knip M, Simell O, Reijonen H. GAD65- and proinsulin-specific CD4+ T-cells detected by MHC class II tetramers in peripheral blood of type 1 diabetes patients and at-risk subjects. *J Autoimmun.* Nov; 2005 25(3):235–243. Epub 2005 Nov 2. [PubMed: 16263242]
43. Pinkse GG, Tysma OH, Bergen CA, Kester MG, Ossendorp F, van Veelen PA, Keymeulen B, Pipeleers D, Drijfhout JW, Roep BO. Autoreactive CD8 T cells associated with beta cell destruction in type 1 diabetes. *Proc Natl Acad Sci U S A.* 2005; 102:18425–18430. [PubMed: 16339897]
44. Pugliese A, Zeller M, Fernandez A Jr, Zalcberg LJ, Bartlett RJ, Ricordi C, Pietropaolo M, Eisenbarth GS, Bennett ST, Patel DD. The insulin gene is transcribed in the human thymus and transcription levels correlated with allelic variation at the INS VNTR-IDD3 susceptibility locus for type 1 diabetes. *Nat Genet.* 1997; 15:293–297. [PubMed: 9054945]
45. von Herrath MG, Dyrberg T, Oldstone MB. Oral insulin treatment suppresses virus-induced antigen-specific destruction of beta cells and prevents autoimmune diabetes in transgenic mice. *J Clin Invest.* 1996; 98:1324–1331. [PubMed: 8823297]
46. Petersen JS, Bregenholt S, Apostolopoulos V, Homann D, Wolfe T, Hughes A, De JK, Wang M, Dyrberg T, von Herrath MG. Coupling of oral human or porcine insulin to the B subunit of cholera toxin (CTB) overcomes critical antigenic differences for prevention of type I diabetes. *Clin Exp Immunol.* 2003; 134:38–45. [PubMed: 12974752]
47. Skyler JS, Krischer JP, Wolfsdorf J, Cowie C, Palmer JP, Greenbaum C, Cuthbertson D, Rafkin-Mervis LE, Chase HP, Leschek E. 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]
48. Pozzilli P, Pitocco D, Visalli N, Cavallo MG, Buzzetti R, Crino A, Spera S, Suraci C, Multari G, Cervoni M, Manca Bitti ML, Matteoli MC, Marietti G, Ferrazzoli F, Cassone Faldetta MR, Giordano C, Sbriglia M, Sarugeri E, Ghirlanda G. 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]
49. Chaillous L, Lefevre H, Thivolet C, Boitard C, Lahlou N, Atlan-Gepner C, Bouhanick B, Mogenet A, Nicolino M, Carel JC, Lecomte P, Marechaud R, Bougneres P, Charbonnel B, Sai P. Oral insulin administration and residual beta-cell function in recent-onset type 1 diabetes: a multicentre randomised controlled trial. *Diabete Insuline Orale group. Lancet.* 2000; 356:545–549. [PubMed: 10950231]
50. Ergun-Longmire B, Marker J, Zeidler A, Rapaport R, Raskin P, Bode B, Schatz D, Vargas A, Rogers D, Schwartz S, Malone J, Krischer J, Maclaren NK. Oral insulin therapy to prevent

- progression of immune-mediated (type 1) diabetes. *Ann N Y Acad Sci.* 2004; 1029:260–277. [PubMed: 15681764]
51. Fouteri G, Chan JR, Zheng Y, Whiting C, Dave A, Bresson D, Croft M, von HM. Virtual optimization of nasal insulin therapy predicts immunization frequency to be crucial for diabetes protection. *Diabetes.* 2010; 59:3148–3158. [PubMed: 20864513]
 52. Harrison LC, Honeyman MC, Steele CE, Stone NL, Sarugeri E, Bonifacio E, Couper JJ, Colman PG. Pancreatic beta-cell function and immune responses to insulin after administration of intranasal insulin to humans at risk for type 1 diabetes. *Diabetes Care.* 2004; 27:2348–2355. [PubMed: 15451899]
 53. Harrison LC. Vaccination against self to prevent autoimmune disease: the type 1 diabetes model. *Immunol Cell Biol.* 2008; 86:139–145. [PubMed: 18180798]
 54. Nanto-Salonen K, Kupila A, Simell S, Siljander H, Salonsaari T, Hekkala A, Korhonen S, Erkkola R, Sipila JJ, Haavisto L, Siltala M, Tuominen J, Hakalax J, Hyoty H, Ilonen J, Veijola R, Simell T, Knip M, Simell O. 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]
 55. Fourlanos S, Perry C, Gellert SA, Martinuzzi E, Mallone R, Butler J, Colman PG, Harrison LC. Evidence that nasal insulin induces immune tolerance to insulin in adults with autoimmune diabetes. *Diabetes.* 2011; 60:1237–1245. [PubMed: 21307076]
 56. Achenbach P, Barker J, Bonifacio E. 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]
 57. Keller RJ, Eisenbarth GS, Jackson RA. Insulin prophylaxis in individuals at high risk of type I diabetes. *Lancet.* 1993; 341:927–928. [PubMed: 8096268]
 58. Effects of insulin in relatives of patients with type 1 diabetes mellitus. *N Engl J Med.* 2002; 346:1685–1691. [PubMed: 12037147]
 59. Gottlieb, Peter, Colman, Peter G., Solvason, Nanette, Otto, Kelly, Roep, Bart, Harrison, Leonard C., Eisenbarth, George, Robinson, William, Utz, Paul J., Steinman, Lawrence, Garren, Hideki, Quan, Joanne. One-Year Results from a Phase 1/2 Clinical Trial of BHT-3021, a DNA Plasmid Vaccine for Type 1 Diabetes (T1D). 70th Scientific Sessions; June 25–29, 2010; Orlando, Florida. 2010.
 60. Bielekova B, Goodwin B, Richert N, Cortese I, Kondo T, Afshar G, Gran B, Eaton J, Antel J, Frank JA, McFarland HF, Martin R. Encephalitogenic potential of the myelin basic protein peptide (amino acids 83–99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. *Nat Med.* 2000; 6:1167–1175. [PubMed: 11017150]
 61. Baekkeskov S, Aanstoot HJ, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, De CP. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature.* 1990; 347:151–156. [PubMed: 1697648]
 62. Velloso LA, Kampe O, Hallberg A, Christmanson L, Betsholtz C, Karlsson FA. Demonstration of GAD-65 as the main immunogenic isoform of glutamate decarboxylase in type 1 diabetes and determination of autoantibodies using a radioligand produced by eukaryotic expression. *J Clin Invest.* 1993; 91:2084–2090. [PubMed: 8486775]
 63. De Aizpurua HJ, Wilson YM, Harrison LC. Glutamic acid decarboxylase autoantibodies in preclinical insulin-dependent diabetes. *Proc Natl Acad Sci U S A.* 1992; 89:9841–9845. [PubMed: 1409709]
 64. Harrison LC, Honeyman MC, DeAizpurua HJ, Schmidli RS, Colman PG, Tait BD, Cram DS. Inverse relation between humoral and cellular immunity to glutamic acid decarboxylase in subjects at risk of insulin-dependent diabetes. *Lancet.* 1993; 341:1365–1369. [PubMed: 8098789]
 65. Hagopian WA, Sanjeevi CB, Kockum I, Landin-Olsson M, Karlsten AE, Sundkvist G, Dahlquist G, Palmer J, Lernmark A. Glutamate decarboxylase-, insulin-, and islet cell-antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. *J Clin Invest.* 1995; 95:1505–1511. [PubMed: 7706455]

66. Solimena M, Folli F, Aparisi R, Pozza G, De CP. Autoantibodies to GABA-ergic neurons and pancreatic beta cells in stiff-man syndrome. *N Engl J Med.* 1990; 322:1555–1560. [PubMed: 2135382]
67. Lohmann T, Hawa M, Leslie RD, Lane R, Picard J, Londei M. Immune reactivity to glutamic acid decarboxylase 65 in stiffman syndrome and type 1 diabetes mellitus. *Lancet.* 2000; 356:31–35. [PubMed: 10892762]
68. De Aizpurua HJ, French MB, Chosich N, Harrison LC. Natural history of humoral immunity to glutamic acid decarboxylase in non-obese diabetic (NOD) mice. *J Autoimmun.* 1994; 7:643–653. [PubMed: 7840856]
69. Tisch R, Yang XD, Singer SM, Liblau RS, Fugger L, McDevitt HO. Immune response to glutamic acid decarboxylase correlates with insulinitis in non-obese diabetic mice. *Nature.* 1993; 366:72–75. [PubMed: 8232539]
70. Kaufman DL, Clare-Salzler M, Tian J, Forsthuber T, Ting GS, Robinson P, Atkinson MA, Sercarz EE, Tobin AJ, Lehmann PV. Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature.* 1993; 366:69–72. [PubMed: 7694152]
71. Jaeckel E, Klein L, Martin-Orozco N, von BH. Normal incidence of diabetes in NOD mice tolerant to glutamic acid decarboxylase. *J Exp Med.* 2003; 197:1635–1644. [PubMed: 12796471]
72. Petersen JS, Karlsen AE, Markholst H, Worsaae A, Dyrberg T, Michelsen B. Neonatal tolerization with glutamic acid decarboxylase but not with bovine serum albumin delays the onset of diabetes in NOD mice. *Diabetes.* 1994; 43:1478–1484. [PubMed: 7958502]
73. Tisch R, Wang B, Weaver DJ, Liu B, Bui T, Arthos J, Serreze DV. Antigen-specific mediated suppression of beta cell autoimmunity by plasmid DNA vaccination. *J Immunol.* 2001; 166:2122–2132. [PubMed: 11160264]
74. Ludvigsson J. Therapy with GAD in diabetes. *Diabetes Metab Res Rev.* 2009; 25:307–315. [PubMed: 19267332]
75. Agardh CD, Cilio CM, Lethagen A, Lynch K, Leslie RD, Palmer M, Harris RA, Robertson JA, Lernmark A. Clinical evidence for the safety of GAD65 immunomodulation in adult-onset autoimmune diabetes. *J Diabetes Complications.* 2005; 19:238–246. [PubMed: 15993359]
76. Agardh CD, Lynch K, Palmer M, Link K, Lernmark A. GAD65 vaccination significantly reduces insulin dependence at five years follow-up in a dose escalating study in adult-onset autoimmune diabetes patients. *Diabetologia.* 2008; 51:S230.
77. Ludvigsson J, Faresjo M, Hjorth M, Axelsson S, Cheramy M, Pihl M, Vaarala O, Forsander G, Ivarsson S, Johansson C, Lindh A, Nilsson NO, Aman J, Orqvist E, Zerhouni P, Casas R. GAD treatment and insulin secretion in recent-onset type 1 diabetes. *N Engl J Med.* 2008; 359:1909–1920. [PubMed: 18843118]
78. Ludvigsson J, Hjorth M, Cheramy M, Axelsson S, Pihl M, Forsander G, Nilsson NO, Samuelsson BO, Wood T, Aman J, Orqvist E, Casas R. Extended evaluation of the safety and efficacy of GAD treatment of children and adolescents with recent-onset type 1 diabetes: a randomised controlled trial. *Diabetologia.* 2011; 54:634–640. [PubMed: 21116604]
79. Hjorth M, Axelsson S, Ryden A, Faresjo M, Ludvigsson J, Casas R. GAD-alum treatment induces GAD65-specific CD4⁺CD25^{high}FOXP3⁺ cells in type 1 diabetic patients. *Clin Immunol.* 2011; 138:117–126. [PubMed: 21044870]
80. Axelsson S, Hjorth M, Ludvigsson J, Casas R. Decreased GAD(65)-specific Th1/Tc1 phenotype in children with Type 1 diabetes treated with GAD-alum. *Diabet Med.* 2012; 29:1272–1278. [PubMed: 22587593]
81. Axelsson S, Cheramy M, Hjorth M, Pihl M, Akerman L, Martinuzzi E, Mallone R, Ludvigsson J, Casas R. Long-lasting immune responses 4 years after GAD-alum treatment in children with type 1 diabetes. *PLoS One.* 2011; 6:e29008. [PubMed: 22174945]
82. Wherrett DK, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R, Gottlieb PA, Greenbaum CJ, Herold KC, Marks JB, Monzavi R, Moran A, Orban T, Palmer JP, Raskin P, Rodriguez H, Schatz D, Wilson DM, Krischer JP, Skyler JS. 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]

83. Ludvigsson J, Krisky D, Casas R, Battelino T, Castano L, Greening J, Kordonouri O, Otonkoski T, Pozzilli P, Robert JJ, Veeze HJ, Palmer J, Samuelsson U, Elding LH, Aman J, Kardell G, Neiderud HJ, Lundstrom G, Albinsson E, Carlsson A, Nordvall M, Fors H, Arvidsson CG, Edvardson S, Hanas R, Larsson K, Rathsmann B, Forsgren H, Desaix H, Forsander G, Nilsson NO, Akesson CG, Keskinen P, Veijola R, Talvitie T, Raile K, Kapellen T, Burger W, Neu A, Engelsberger I, Heidtmann B, Bechtold S, Leslie D, Chiarelli F, Cicognani A, Chiumello G, Cerutti F, Zuccotti GV, Gomez GA, Rica I, Barrio R, Clemente M, Lopez Garcia MJ, Rodriguez M, Gonzalez I, Lopez JP, Oyarzabal M, Reeser HM, Nuboer R, Stouthart P, Bratina N, Bratanic N, de KM, Weill J, Ser N, Barat P, Bertrand AM, Carel JC, Reynaud R, Coutant R, Baron S. GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus. *N Engl J Med*. 2012; 366:433–442. [PubMed: 22296077]
84. Elias D, Markovits D, Reshef T, van der Zee R, Cohen IR. Induction and therapy of autoimmune diabetes in the non-obese diabetic (NOD/Lt) mouse by a 65-kDa heat shock protein. *Proc Natl Acad Sci U S A*. 1990; 87:1576–1580. [PubMed: 2406723]
85. Elias D, Reshef T, Birk OS, van der Zee R, Walker MD, Cohen IR. Vaccination against autoimmune mouse diabetes with a T-cell epitope of the human 65-kDa heat shock protein. *Proc Natl Acad Sci U S A*. 1991; 88:3088–3091. [PubMed: 1707531]
86. Elias D, Cohen IR. Peptide therapy for diabetes in NOD mice. *Lancet*. 1994; 343:704–706. [PubMed: 7907681]
87. Elias D, Marcus H, Reshef T, Ablamunits V, Cohen IR. Induction of diabetes in standard mice by immunization with the p277 peptide of a 60-kDa heat shock protein. *Eur J Immunol*. 1995; 25:2851–2857. [PubMed: 7589082]
88. Abulafia-Lapid R, Elias D, Raz I, Keren-Zur Y, Atlan H, Cohen IR. T cell proliferative responses of type 1 diabetes patients and healthy individuals to human hsp60 and its peptides. *J Autoimmun*. 1999; 12:121–129. [PubMed: 10047432]
89. Raz I, Elias D, Avron A, Tamir M, Metzger M, Cohen IR. 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]
90. Zanin-Zhorov A, Cahalon L, Tal G, Margalit R, Lider O, Cohen IR. Heat shock protein 60 enhances CD4⁺ CD25⁺ regulatory T cell function via innate TLR2 signaling. *J Clin Invest*. 2006; 116:2022–2032. [PubMed: 16767222]
91. Hurman VA, Decochez K, Mathieu C, Cohen IR, Roep BO. Therapy with the hsp60 peptide DiaPep277 in C-peptide positive type 1 diabetes patients. *Diabetes Metab Res Rev*. 2007; 23:269–275. [PubMed: 17024692]
92. Lazar L, Ofan R, Weintrob N, Avron A, Tamir M, Elias D, Phillip M, Josefsberg Z. 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]
93. Schloot NC, Meierhoff G, Lengyel C, Vandrofi G, Takacs J, Panczel P, Barkai L, Madacsy L, Oroszlan T, Kovacs P, Suto G, Battelino T, Hosszufalusi N, Jermendy G. Effect of heat shock protein peptide DiaPep277 on beta-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]
94. Buzzetti R, Cernea S, Petrone A, Capizzi M, Spoletini M, Zampetti S, Guglielmi C, Venditti C, Pozzilli P. C-peptide response and HLA genotypes in subjects with recent-onset type 1 diabetes after immunotherapy with DiaPep277: an exploratory study. *Diabetes*. 2011; 60:3067–3072. [PubMed: 21896927]
95. Bresson D, Togher L, Rodrigo E, Chen Y, Bluestone JA, Herold KC, von HM. Anti-CD3 and nasal proinsulin combination therapy enhances remission from recent-onset autoimmune diabetes by inducing. *Tregs, J Clin Invest*. 2006; 116:1371–1381. [PubMed: 16628253]
96. Getts DR, Martin AJ, McCarthy DP, Terry RL, Hunter ZN, Yap WT, Getts MT, Pleiss M, Luo X, King NJ, Shea LD, Miller SD. Microparticles bearing encephalitogenic peptides induce T-cell tolerance and ameliorate experimental autoimmune encephalomyelitis. *Nat Biotechnol*. Dec; 2012 30(12):1217–1224. <http://dx.doi.org/10.1038/nbt.2434> (Epub 2012 Nov 18). [PubMed: 23159881]

97. Clemente-Casares X, Tsai S, Yang Y, Santamaria P. Peptide-MHC-based nanovaccines for the treatment of autoimmunity: a “one size fits all” approach? *J Mol Med (Berl)*. 2011; 89:733–742. [PubMed: 21499734]
98. Luo X, Pothoven KL, McCarthy D, DeGutes M, Martin A, Getts DR, Xia G, He J, Zhang X, Kaufman DB, Miller SD. ECDI-fixed allogeneic splenocytes induce donor-specific tolerance for long-term survival of islet transplants via two distinct mechanisms. *Proc Natl Acad Sci U S A*. 2008; 105:14527–14532. [PubMed: 18796615]
99. Vandemeulebroucke E, Gorus FK, Decochez K, Weets I, Keymeulen B, De BC, Tits J, Pipeleers DG, Mathieu C. Insulin treatment in IA-2A-positive relatives of type 1 diabetic patients. *Diabetes Metab*. 2009; 35:319–327. [PubMed: 19647467]

Figure 1.

Staging of clinical trials in type 1 diabetes. The top panel shows the different disease stages with corresponding rates of beta cell mass decline and associated trial terminology. Lower panel indicates the fact that prevention trials are more costly, take more time but have a more clinically relevant endpoint (diabetes prevention) than intervention trials (C-peptide preservation). Intervention trials tend to offer a more stringent therapeutic setting yet are associated with easier recruitment and higher patient motivation.

Figure 2.

Antigen-specific tolerance induction and the concept of linked suppression. The mucosal immune system has the unique capacity to propagate the induction of antigen-specific regulatory T cells (Treg). These Treg subsequently regulate immune responses as shown here in the pancreatic draining lymph nodes. In type 1 diabetes, islet antigens are constantly drained from the pancreas by antigen-presenting cells (APC). Treg subsequently recognize the tolerizing antigen (here antigen A) and promote a regulatory response. Linked suppression would then account for a similar response to an unrelated antigen that is presented by the same APC (here antigen B). Treg are known to act via suppressive cytokines or contact-dependent mechanisms on both other T cells and APC.

Table 1

Antigen-specific therapies described in this review article. This article almost solely focusses on larger controlled trials, some small proof-of-concept trials are included that are of particular interest. The trials are listed according to their order of appearance in this article.

Trial designation+ref.	Design	Outcome
<i>(prepro)Insulin</i>		
Diabetes Prevention Trial (DPT-1) [47] (oral insulin)	Secondary prevention	No delay, post hoc subgroup effect identified
NCT00419562 (oral insulin)	Secondary prevention	Currently recruiting, results expected February 2014
IMDIAB [48]	Recent-onset	No preservation of beta cell function
Diabète Insuline Orale group [49]	Recent-onset	No preservation of beta cell function
MacLaren et al. [50]	Recent-onset	No preservation of beta cell function
Type 1 diabetes prediction and prevention (DIPP) [54] (nasal insulin)	Secondary prevention	No delay
Intranasal insulin trial (INIT-1) [52]	Safety trial in prevention	Safe, no delay or acceleration, INIT-II trial ongoing (NCT00336674)
Fourlanos et al. [55]	Recent-onset	No preservation of beta cell function
Primary Oral/intranasal INSulin Trial (Pre-point) [56] (oral/nasal insulin)	Primary prevention	Recruiting
Diabetes Prevention Trial (DPT-1) [58] (parenteral insulin)	Secondary prevention	No delay
Belgian Diabetes Registry [99] (parenteral insulin)	Secondary prevention	No delay
Proinsulin peptide vaccination [36]	Safety trial in longstanding disease	Safe, immunological tolerance signs observed
Insulin B chain vaccination [37]	Safety trial in recent-onset	Safe, immunological tolerance signs observed
<i>GAD65</i>		
GAD-alum [75,76]	Dose-escalation study in LADA	Safe, beta cell function preservation with 20 µg dose
GAD-alum [77]	Recent-onset	Beta cell function preservation
GAD-alum (TrialNet) [82]	Recent-onset	No preservation of beta cell function
GAD-alum (Europe) [83]	Recent-onset	No preservation of beta cell function
GAD-alum (U.S., DIAPREVENT, NCT00751842)	Recent-onset	Suspended
Diabetes prevention — Immune Tolerance (DIAPREV-IT; NCT01122446)	Secondary prevention	Ongoing, fully enrolled
<i>HSP60 (p277)</i>		
DiaPep277 pilot trial [89]	Recent-onset	Evidence of beta cell preservation
Huurman et al. [91]	Recent-onset	Trend for beta cell preservation
Lazar et al. [92]	Recent-onset	No trend for beta cell preservation
Schloot et al. [93]	Recent-onset	Trend for beta cell preservation
Buzzetti et al. [94]	Recent-onset	Trend for beta cell preservation
DiaPep277 phase III trial (www.andromedabio.com/news.php)	Recent-onset	Evidence of beta cell preservation, confirmatory trial ongoing