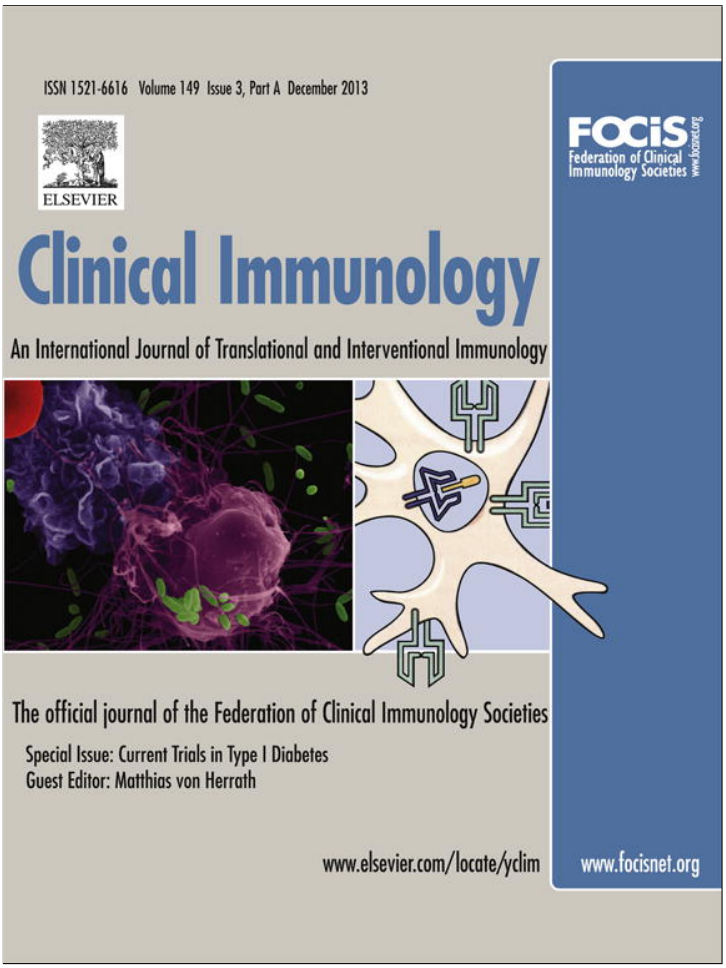

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REVIEW

DiaPep277® and immune intervention for treatment of type 1 diabetes

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Abstract Type 1 diabetes is a chronic immune-mediated disease resulting in destruction of insulin-producing β-cells. Several studies have been performed aiming to halt disease progression after diagnosis; to reduce the increased diabetes risk in islet-autoantibody positive subjects; and to prevent the onset of β-cell autoimmunity in subjects genetically at risk but without autoantibodies. Whereas secondary prevention trials failed, trials in newly diagnosed patients have shown partial success in preserving C-peptide. These studies target T-cells and inflammation and make use of antigen-specific immune modulation or stem cell approaches. However, thus far no immune-based therapeutic regimen has cured type 1 diabetes after its clinical onset or has stabilized the decline of C-peptide to achieve the status of an approved drug. This review summarizes immune intervention trials and the current knowledge of DiaPep277® peptide as a form of immune intervention in type 1 diabetes.

Contents

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1. Introduction

Type 1 diabetes accounts for about 10% of all diabetes, affecting approximately 1.4 million people in the U.S., and 10–20 million globally [1,2]. About 40% of persons with type 1 diabetes develop the disease before 20 years of age, thus making it one of the most common severe chronic diseases of childhood [2]. In adulthood, about 10% of patients have type 1 diabetes or LADA (latent autoimmune diabetes of the adult), which is clinical type 2 diabetes with positive islet-reactive autoantibodies [3]. T cells and cellular immune reactivity play a crucial role in β-cell destruction leading to insulin deficiency and the necessity to treat these patients lifelong with insulin. β-Cell loss starts without symptoms at an unknown time before clinical onset and continues thereafter.

Worldwide, the incidence of type 1 diabetes varies. For example the incidence in subjects under 15 years is <0.1 per 100,000 per year in China, rising to 40 per 100,000 per year in Finland [4]. In recent decades the incidence in Finland and Germany has risen, increasing yearly by 5% with a predominance in small children [5]. As this rapid development cannot be explained by genetic factors, environmental changes are thought to account for the rise in incidence. It is reasonable to assume that less susceptible genotypes are sufficient to facilitate the autoimmune process of β-cell destruction [6]. Modified diet, hygiene and altered microbiota are discussed as important potential triggers. However these epidemiological studies cannot prove causality [7].

2. Treatment of type 1 diabetes with insulin

For more than 90 years insulin has been the only medication available to efficiently treat and save subjects suffering from type 1 diabetes. Data from the Diabetes Control and Complication study (DCCT) in the 1980s established that good glycemic control, often achieved by intensified insulin treatment as a golden standard, not only prevents acute symptoms but also reduces the occurrence of diabetes-associated complications resulting from microangiopathy (nephropathy, retinopathy, neuropathy) and macroangiopathy (coronary disease, stroke, peripheral arterial disease).

Data from the DCCT also showed that increased or stabilized C-peptide as a marker of endogenous insulin secretion in type 1 diabetes is associated with fewer diabetes-associated complications including hypoglycemia, thereby indicating that preservation of C-peptide, even if minor, is of benefit for the patient with type 1 diabetes [8]. Similarly, good glycemic control when achieved after diabetes onset can preserve endogenous insulin secretion and should be a goal of treatment in newly diagnosed patients with type 1 diabetes. Patients with hypoglycemic unawareness often have no residual C-peptide and benefit from islet transplantation, which can reduce insulin requirements but also markedly improve hypoglycemic awareness [9]. Because of these observations, it would be desirable to preserve or improve endogenous insulin secretion measured by C-peptide in patients with type 1 diabetes, when they are in need of continuing exogenous insulin treatment [10].

3. Prevention of β-cell loss in prediabetic islet antibody-positive subjects

Secondary prevention trials are performed in subjects who are islet antibody positive and are therefore at increased risk of developing type 1 diabetes. These studies require large-scale screening, as antibody-positive subjects are rarely found in the general population. Accordingly most of these studies screen and recruit first-degree relatives of type 1 diabetes patients. Still, several thousand to ten thousand subjects need to be screened before sufficient subjects could be enrolled for secondary prevention trials.

The diabetes prevention trial (DPT-1) performed in the US in the 1990s aimed at halting progressive β-cell failure before the onset of clinical diabetes by injecting insulin. This trial failed for reasons not well understood, perhaps a lack in the optimal route of insulin administration, dose or timing [11]. However, the oral insulin arm of the DPT-1 showed promising results, namely an increase in insulin secretory capacity especially in the subgroup of insulin autoantibody-positive (mIAA) subjects [12].

Studies with intra-nasally administered insulin in subjects genetically at risk who converted to being antibody positive failed too [13]. Nevertheless, the latter study with intranasal insulin lispro is now being repeated with higher dosing and studies such as PrePoint and Point using oral insulin treatment in subjects at increased risk of diabetes are underway [14]. Other secondary intervention trials, such as ENDIT performed in Europe, treated pre-diabetic islet antibody positive patients with nicotinamide also failed to delay or prevent onset of type 1 diabetes [15].

Although it is reasonable to believe that secondary prevention should be easier to achieve than tertiary prevention as there are more intact β-cells left, the mild therapeutic regimens used thus far have failed to halt β-cell deterioration.

Indeed, there is a reluctance to try more aggressive treatment as potential side effects are unacceptable in apparently healthy, non-diabetic subjects who are often at a young age.

4. Immune intervention studies in type 1 diabetes

The primary goal of these studies is preservation of C-peptide, as a measure of arrest or decrease in continuing β-cell destruction, or in the best case, improvement of C-peptide as a sign of recovered β-cell function. C-peptide can be measured from fasting blood samples or upon stimulation with either glucagon 1 mg intravenously or a standardized mixed meal test (MMTT) [16]. Although improved β-cell function should be associated with improved glycemic control as measured by glycated hemoglobin (HbA1c) and lower insulin requirements, it is recommended not to use HbA1c alone as a primary readout for such trials. A high glucose concentration is in itself toxic to β-cells and hyperglycemia should be minimized; therefore patients should be treated with insulin to optimally protect them from glucotoxicity in the placebo as well as in the test treatment arms.

Most tertiary prevention trials have been performed in newly diagnosed type 1 diabetes patients diagnosed within 3–6 months after detection of hyperglycemia. The major advantages of treating diagnosed patients are that such patients are easy to identify and they are clearly in need of treatment. However, the success of these trials is limited since β-cell function has already decreased to a much greater extent than found in pre-diabetic, normoglycemic subjects.

Several different approaches have been tried to halt β-cell destruction. They can be grouped into *anti-inflammatory*, *immune-cell directed* and *antigen-specific* approaches [17]. All of them have shown promising results in preclinical studies such as the non-obese diabetic (NOD) mouse or other animal models.

4.1. Anti-inflammatory and cytokine inhibition approaches

Since β-cell destruction results from an inflammatory process and pro-inflammatory cytokines such as TNF-α or IL-1 are cytotoxic to β-cells, blockade of inflammation seems to be a logical treatment option to counteract this process. In one small study, 18 children (age 3–18 years, with diabetes diagnosed less than 5 weeks earlier) were treated with anti-TNF-α antibodies (Etanercept) or placebo over 24 weeks [18]. Indeed, C-peptide secretion could be stabilized with Etanercept, and HbA1c was improved in the verum treated patients. Although at first sight this seems beneficial, HbA1c differences in placebo compared to treatment are associated with different degrees of glucotoxicity making it difficult to judge whether the improved C-peptide resulted from arrest of immune-mediated destruction or primarily from improved glucose control. Confirmatory studies at a larger scale are required given these results and the small study size.

In another study, recent onset (less than 6 weeks) type 1 diabetes patients (age 3–25 years) were treated orally for one year with recombinant IFN-α (5000 or 30,000 units daily). The results showed a slower decrease in the treatment group (5000 units) than in the placebo group or in the treatment group receiving 30,000 units [19].

In a third study, 89 adult type 1 diabetes patients (age 18–39, diabetes duration <3 months) were treated with atorvastatin compared to placebo [20]. Atorvastatin is known for its therapeutic effects on cholesterol and for its anti-inflammatory properties [21]. After 12 months of treatment both treatment arms showed a decline in C-peptide. In the atorvastatin group, however, C-peptide thereafter was stabilized whereas a further decline was observed in the placebo group. Interestingly, the subgroup defined by high (above median) baseline C-reactive protein (CRP) concentrations exhibited higher stimulated C-peptide secretion after statin treatment compared to placebo, and individual baseline CRP levels correlated with C-peptide outcome in the statin group [22]. Currently a study is testing atorvastatin in the pediatric age group (ClinicalTrials.gov identifier: NCT00529191).

Given the beneficial effects of IL-1 receptor antagonist (IL-1RA, Anakinra, Kineret) in the treatment of patients with type 2 diabetes [23] or with rheumatoid arthritis, IL-1 blockade with IL1RA might also benefit type 1 diabetes patients. This idea was supported: IL-1 is cytotoxic to β cells in vitro, and systemic IL-1RA in peripheral blood samples is positively associated with β-cell function and remission in recent onset type 1 diabetes [24,25]. Two multicenter studies tried this approach in children and adults with newly diagnosed type 1 diabetes [26]. Unfortunately in neither age group was amelioration of C-peptide loss achieved with blockade of IL-1; the reasons might have been suboptimal dosing regimens or timing.

4.2. Immune cell directed approaches

4.2.1. T-cell targeted therapy

As CD3-expressing T-cells are considered major players in β-cell destruction, treatment with anti-CD3 looked promising through several studies in human subjects. Trials with the anti-CD3 antibodies Teplizumab and Otelixizumab showed that endogenous C-peptide could be stabilized. In addition, they showed reduced insulin requirements despite similarly good HbA1c [27,28]. Especially with Otelixizumab side effects were considerable showing flu-like symptoms, rash, and reactivation of latent EBV infection. Still, patients treated with Otelixizumab for 8 days intravenously at a relatively high dose maintained their improved β-cell function for at least 4 years.

In view of the undesirable side effects induced by the previously tested doses, a subsequent phase 3 study DEFEND-2 applied a dose of 1/16th and the side effects almost vanished but the stabilization of C-peptide also disappeared.

When Teplizumab was tested in phase 3 studies (Protégé study), the primary endpoints chosen, HbA1c and insulin requirement, were not improved compared to placebo and the study was defined to be unsuccessful on this basis [29]. Although formally correct, HbA1c is not an ideal surrogate in studies where glucotoxicity is involved. C-peptide meta-analysis data of this study showed a benefit from Teplizumab treatment and a recent phase II study in patients (age 8–30 years) with

type 1 diabetes diagnosis for 4–12 months showed preservation of β-cell function and the rates of β-cell loss were reduced significantly compared to placebo [30].

4.2.2. B-cell directed therapy

In human type 1 diabetes, islet-directed antibodies secreted by B-cells accompany islet-cell destruction and are used as a gold standard to estimate the risk of developing type 1 diabetes or to confirm the diagnosis of type 1 diabetes. A single case study, however, showed that a patient with a B-cell deficiency could still develop type 1 diabetes [31]. Therefore, B cells have been considered to be not as important as T cells in the pathogenesis of type 1 diabetes. Surprisingly, a study with Rituximab, directed against B cells in patients (n =87, age 8–40 years) showed transient stabilization of C-peptide when compared to placebo. Similar to some studies reported above, the difference of HbA1c in placebo and treated patients makes it difficult to interpret these interesting data [32].

4.2.3. Antigen specific therapy

Islet antigen-reactive T cells play a major role in β-cell destruction and islet-antigen specific therapy has been successfully performed in animal models. Depending on the form of application (oral, intravenous, subcutaneous, intramuscular) and the addition of adjuvants, autoimmune diabetes can either be accelerated or ameliorated. In type 1 diabetes GAD65 and proinsulin are major targets of the adaptive immune response.

Antigen-specific therapy with GAD, 20 μg administered subcutaneously two times after diabetes diagnosis led to stabilized C-peptide levels in patients with type 1 diabetes [33]. Thirty months thereafter, improved C-peptide concentrations were still seen in verum treated patients. However, a multicentric phase III study in patients failed to reproduce these promising results [34] and it remains to be seen which subgroups could benefit from such a treatment.

Another promising study has been performed with the proinsulin plasmid BHT-3021 [35]. DNA plasmid encoding proinsulin or placebo was given weekly for 12 weeks intramuscularly to patients diagnosed with type 1 diabetes within the past 5 years (age above 18 years). The study demonstrated that this treatment reduced the frequency of CD8(+) T cells reactive to proinsulin while preserving C-peptide over the course of dosing without serious side effects [35].

5. DiaPep277® treatment of recent-onset T1D: a novel physiological therapy

DiaPep277® is a 24-amino acid peptide derived from the 437–460 sequence of the human 60 kD heat shock protein (HSP60). The peptide was first discovered to arrest the progression of β-cell destruction in NOD mice [36] and was effective in mice even in tertiary prevention after clinical onset [37]. Subsequent research has shown that, the HSP60 parent molecule and its DiaPep277® fragment both function in a regulatory network intrinsic to the immune system [38]. Immune responsiveness to HSP60 and its peptide epitopes is present in healthy humans from birth: human cord blood contains a high frequency of antibodies and T cells reactive to HSP60 epitopes [39,40] suggesting that responsiveness to DiaPep277® is built into the immune system. In line with these observations, natural antibodies to HSP60 and to DiaPep277® have been detected in NOD mice that resist T1D, even when they have not been treated with exogenous DiaPep277® or HSP60 [41,42]. Thus, effective treatments of autoimmune diabetes in mouse models that are not based on HSP60 therapy also appear to activate p277 antibodies. DiaPep277® treatment also arrests autoimmune diabetes induced by low-dose streptozotocin in otherwise normal mice [43].

The phenotype of immune modulation in T1D by DiaPep277® appears to be unique in manifesting a limited anatomic expression: Islet-infiltrating T cells in treated NOD mice lose their secretion of pro-inflammatory IFNγ in response to mitogenic anti-CD3 activation. However IFNγ is secreted in response to mitogenic stimulation in the spleens of these same mice [44]. Thus, the anti-inflammatory effects of DiaPep277® treatment appear to be limited to the site of the autoimmune process.

The immune modulation induced by DiaPep277® treatment appears to discriminate between diabetes-associated autoimmunity and immunity to foreign antigens: DiaPep277® therapy induces down-regulation of Th1 effector autoimmunity and activates a Th2 cytokine switch in T cells responding to insulin, to GAD and to whole HSP60, as well as to DiaPep277®. Th1 effector T cell immunity to bacterial antigens and other recall antigens is not affected and remains intact (for mice see: [45]; for a human phase 2 trial see [46]). Thus, the cytokine switch induced by DiaPep277® treatment spreads to cover a range a self-antigens targeted in β-cell destruction, but does not cross the lines to affect immune responses to foreign antigens.

The unique immune regulatory effects of DiaPep277® can be attributed to the finding that the peptide is a ligand for the innate TLR-2 receptor on Tregs and other T cells, as well as an antigen for antigen receptors on T cells and B cells [47,48]. The parent HSP60 molecule too is both an antigen for T and B cells and an innate ligand for TLR-4 on macrophages, dendritic cells and B cells and for TLR-2 on T cells. Note however, that the whole HSP60 molecule can induce either pro-inflammatory or anti-inflammatory modulation depending on the concentration of HSP60 and the cells it interacts with. However, the p277 peptide that forms DiaPep277® has only anti-inflammatory influences. The effects of p277 as an enhancer of Tregs are an especially important in DiaPep277® treatment [47]. The p277 peptide also modulates T-cell homing to inflammatory sites [48] and induces the Th2 transcription factor SOCS3.

We hypothesize that the integration of these various interactions of DiaPep277® within the immune system ultimately leads to arrest of the diabetogenic destruction of β-cells. Note, that these effects can be viewed as physiological in the sense that the immune system is outfitted with both adaptive and innate receptors on a variety of immune system cells poised to sense the concentration of peptide p277. DiaPep277® treatment is unique in that it communicates, metaphorically, with the immune system in its own molecular language. This hypothesis, of course, will be rejected or validated as studies progress. In fact, although early treatment results with DiaPep277® look promising, we do not know yet whether continued treatment will have a beneficial effect on β-cell deterioration in the long term.

5.1. Human phase Ib and phase II clinical trials with DiaPep277®

5.1.1. Safety and tolerability of DiaPep277® treatment

To date more than 250 patients have received repeated doses of DiaPep277® in clinical phase I and II trials, and several hundreds more have received multiple doses of DiaPep277® in phase III trials (Table 1). The safety and tolerability of DiaPep277® have been monitored through vital signs, ECG, laboratory data and adverse event reports in children and adults. No drug- or dose-dependent differences between groups with regard to serious adverse events, laboratory abnormalities or changes in vital signs have been reported. The adverse events reported in DiaPep277® clinical studies were similar in adults with T1D or LADA and in children or adolescents with T1D also treated with the placebo.

Across all completed studies, there were no reports of specific immune system disorders including autoimmune diseases. The safety profile of DiaPep277®, thus far, is similar to that of placebo, and no increase in infections or malignancies was reported. However there was a slight increase in local reactions after injection using DiaPep277® versus placebo [49]. As we know, however, any treatment with the power to help bears the potential to harm; there is no free lunch, as the saying goes. The cost–benefit ratio will emerge as clinical use proceeds.

5.1.2. Outcome of phase II trials with DiaPep277® treatment (Table 1)

5.1.2.1. Studies in adults. The results of the first small-size clinical, randomized, double-blind study were published in 2001 [46]. Thirty-five patients, aged 16–65 years who had been diagnosed with type 1 diabetes for less than 6 months were included. Patients were positive for autoantibodies to glutamic acid decarboxylase or IA–2A (ICA512) or had hyperglycemia with ketonuria or were under 25 years old and manifested residual beta-cell function with a basal C-peptide concentration of more than 0.1 nmol/l. DiaPep277® was given subcutaneously at study start, after 1 month and after 6 months. C-peptide was evaluated in response to glucagon stimulation and showed a consecutive decrease over 10 months in the placebo group and a relative preservation or increase that was statistically significant in the DiaPep277® group. At 10 months, stimulated C-peptide was more than twice as high in the DiaPep277® group than in the placebo group. Interestingly, this was observed despite the fact that patients from the placebo group had started with higher C-peptide levels than those who were randomized to receive DiaPep277® [46].

A follow-up study was carried on these 35 male patients for an additional year [50]. After 18 months stimulated C-peptide concentrations had fallen in the placebo group but were maintained in the DiaPep277® group ($P = 0.0005$). The need for exogenous insulin was higher in the placebo group than in the DiaPep277® group. Mean HbA1c concentrations were similar in both groups. After extension of the study, patients continuing treatment with DiaPep277® and those switched from placebo to DiaPep277® manifested a trend towards a greater preservation of beta-cell function compared to patients maintained on or switched to placebo. The authors concluded that periodic treatment of subjects with DiaPep277® over 2 years was safe and associated preservation of endogenous insulin secretion up to 18 months was observed.

Two further trials were performed in adult patients in Belgium and Hungary. In the Belgium study [51] forty-eight patients were recruited within 42 months of diagnosis; were aged 18–45 years; manifested remaining basal C-peptide concentrations of >0.1 nmol/l; and were positive for islet autoantibodies (ICA, GAD and/or IA-2-antibody). Patients were assigned to subcutaneous injections of 0.2, 1.0 or 2.5 mg peptide DiaPep277® ($n = 12$ per dosage) at entry, and 1, 6 and 12 months, or four placebo injections ($n = 12$). C-peptide levels were measured by the area under the curve following stimulation with glucagon. The C-peptide decreased over time in all groups, but the decrease in C-peptide production was less in treated patients versus placebo, mostly in the 2.5 mg group. Both the 0.2 mg and the 1.0 mg group showed less of a decrease than did the placebo group, but these differences were not statistically significant. All treated groups combined showed significantly less decrease after 12 months than did the placebo group ($P < 0.05$). Secondary endpoints HbA1c and insulin dosage were unaffected.

The second study included 50 young adults from Hungary (aged 16–45 years) who were diagnosed with type 1 diabetes for less than 3 months [52]. They were treated subcutaneously at four different time points (baseline, 1, 6, 12 months) with 0.2 mg (n = 12), 1.0 mg (n = 12), and 2.5 mg (n = 13) DiaPep277® versus placebo (n = 13) and followed for 18 months. As in the other studies, these patients had to be diabetes related autoantibody positive and manifest C-peptide concentrations above 0.25 nmol/l. Glucagon-stimulated C-peptide served as the readout for functional β-cell mass. A modest trend towards better maintenance of β-cell function was observed in the 0.2 mg and 1.0 mg group, while there was significant loss of stimulated C-peptide in the placebo and 2.5 mg group. Thus, the Hungarian study compared to the study from Belgium, differed in the dosage associated with improved C-peptide. Secondary endpoints of HbA1c and insulin dosage were not different between the placebo and treatment groups at baseline or 18 months compared to placebo.

A meta-analysis pooled patients data from the above studies and showed that adult patients who benefited the most from intervention with DiaPep277® were those who bore low or moderate risk HLA genotypes [53]. The only subgroup with an increase of C-peptide at 12 months after diagnosis was the DiaPep277®-treated subgroup with a low-risk genotype. DiaPep277®-treated adults with a low-risk genotype manifested significantly higher maximal and AUC C-peptide versus placebo at 12 months (0.04 ± 0.07 vs. -0.28 ± 0.09 nmol/l, $P < 0.01$; and 0.53 ± 1.3 vs. -4.59 ± 1.5 nmol/l, $P < 0.05$, respectively). In patients with moderate-risk genotypes, the change over the study in the maximal and AUC C-peptide values after stimulation with glucagon was significantly higher in the DiaPep277®-treated versus placebo-treated patients ($P < 0.01$ and $P < 0.05$, respectively) [53].

5.1.3. Studies in LADA/adult autoimmune diabetes

Two additional phase II studies were carried out in adult autoimmune diabetes patients aged 30–65 years who were

Table 1 Clinical studies with DiaPep277®.

Subjects/sites	Phase	Age	Inclusion criteria	Subjects enrolled	Dose(s)	Dosing regimen	Outcome	Reference
Adult, United Kingdom	1	18–50s	Male. T1D >2 years < 20 years	16	0.1, 0.5 or 2.5 mg	0, 6 months	The peptide was found to be safe and well-tolerated in patient volunteers with established T1D	In file
Adult, Belgium	2	18–45	T1D > 6 months < 42 months. Diabetes related autoantibodies. Fasting CPT > 0.1 nmol/l	48	0.2 or 1.0 or 2.5 mg	0, 1, 6, 12 months	Good safety data. Better glucagon stimulation CPT at 12 months in 2.5 mg DiaPep277 group than in placebo group	[60]
Adult, Hungary	2	16–45	T1D < 3 months. Diabetes related autoantibodies. Fasting CPT > 0.25 nmol/l	50	0.2 or 1.0 or 2.5 mg	0, 1, 6, 12 months	Good safety data better glucagon stimulated CPT at 18 months in 0.2 mg and 1.0 mg DiaPep277® groups vs placebo	[52]
Adult, Israel	2	16–65	T1D < 6 months. Fasting CPT > 0.1 ng/ml	35	1.0 mg	0, 1, 6, 12 months	Good safety data better glucagon stimulated CPT at 10 months in DiaPep277® group and maintenance at 18 months compared to placebo	[46]
Pediatric, Israel	2	4–18	T1D, 8 months Fasting C-peptide > 0.1 ng/ml	30	1.0 mg	0, 1, 6, 12 months	Good safety data. No difference between treatment arms	[50]
Pediatric, Slovenia	2	4–16	T1D < 3 months. Fasting CPT > 0.3 nmol/l	49	0.2 or 1.0 mg	0, 1, 6, 12 months	Good safety data. No difference between the treatment arms. Some benefit for 1 mg DiaPep277® group with HLA low risk	[52]
LADA, Europe	2	30–50	LADA for 2 months to 5 years. $GADA^{+ve}$ and fasting CPT > 0.3 nmol/l	41	1.0 mg 1.0 mg 0.2 mg	0, 4, 26, 52, 78 weeks 0, 2, 4, 6, 8, 21, 34, 48, 60, 78 weeks 0, 2, 4, 6, 8, 26, 28, 30, 32, 34, 52, 54, 56, 58, 60, 78, 80, 82, 84, 86	Good safety data. Unpublished C-peptide data.	[54]
LADA, USA	2	30–65	LADA for 2 months to 5 years. $GADA^{+ve}$ and fasting CPT > 0.3 nmol/l	96	1.0 mg	0, 1, 3, 6, 9, 12, 15, 18 months	Good safety data. Unpublished C-peptide data.	[54]
Adult, 46 sites	3	16–45	T1D < 3 months. Diabetes related autoantibodies. Fasting CPT > 0.22 nmol/l	457	1.0 mg (3-months follow up)	0, 1, 3, 6, 9, 12, 15, 18, 21 months	Manuscript submitted	[62]
Adult, 100 sites	3	20–45	T1D < 6 months. Diabetes related autoantibodies. Fasting CPT > 0.22 nmol/l	475	1.0 mg	0, 3, 6, 9, 12, 15, 18, 21, 24 months	Ongoing-study completion October 2014	

positive for antibodies to GAD; such patients have been termed LADA or SPIDDM patients. One study with 96 patients was carried out in the US (ClinicalTrials.gov identifier: NCT000 58981); the other recruited 41 subjects in Europe (Italy, UK, Germany) [54]. In the US study, diabetes duration was more than 2 months and less than 5 years, and the patients were controlled by diet and insulin (plus metformin, if needed) prior to baseline visit. The C-peptide level was 0.30 nmol/l or greater, or 0.90 ng/ml at the time of the Screen Visit. Treatment was administered at time 0, 1 and 3 months, and then every 3 months for a total of 8 administrations. The total duration of the trial was 24 months (treatment for 18 months and follow-up for an additional 6 months); the results, unfortunately, have not yet been published. A brief report suggests good safety and tolerability to DiaPep277® in the 96 US and 41 European patients treated with 1 mg doses of DiaPep277® [45,40] and personal communication). However, β-cell function was not significantly influenced upon treatment with DiaPep277® in either direction in these patients.

5.1.4. Studies in children

Type 1 diabetes is a major problem in children. Two studies were performed with DiaPep277® in young age groups. A study performed in Israel included 30 children (19 males) aged 7–14 years who had been diagnosed with type 1 diabetes from 53 to 116 days earlier, and manifested basal C-peptide concentrations above 0.1 nmol/l [55]. The children were randomized to receive subcutaneous injections of 1 mg DiaPep277® (15 patients) or 40 mg mannitol (placebo) at entry and at 1, 6, and 12 months and were followed over 18 months. C-peptide levels similarly decreased over time in the DiaPep277®- and placebo-treated patients. There was no significant difference in insulin dose or HbA1c concentration between the groups at any time point. The authors concluded that one-year treatment with DiaPep277® at a dosage of 1 mg is safe for use and well tolerated in children with recent-onset T1DM. However, it appeared to have no beneficial effect in preserving beta-cell function or improving metabolic control.

In another study [52], 49 pediatric patients from Slovenia (4–14 years of age) with type 1 diabetes and basal C-peptide concentrations above 0.2 nmol/l were assigned injections of 0.2 mg (n = 16), 1 mg (n = 17) DiaPep277® or placebo (n = 16) at entry, 1 month, 6 months, and 12 months. Children were followed for 18 months. Pediatric patients bearing low-risk HLA genotypes showed stable C-peptide levels over 13 months upon treatment with 1 mg DiaPep277®. However, no beneficial treatment effect with DiaPep277® was observed when analyzing C-peptide data irrespective of HLA. Despite similar stimulated C-peptide levels at baseline, children exhibited a more pronounced loss of β-cell function over 18 months than did adults from the Hungarian study (P = 0.0003). Younger patients manifest a more accelerated course of β-cell loss than do adults, and earlier treatments may be required when using immune modulators such as DiaPep277®; HLA genotype might also need to be taken into account when designing optimal dose and dose scheduling.

5.1.5. Phase III trials

One phase III trial of DiaPep277® treatment has been completed and has been submitted for publication, but the paper has not yet been accepted as of this writing.

In 40 clinical centers in 3 continents, 457 newly diagnosed type 1 diabetes patients, aged 16–45 years, manifesting fasting C-peptide ≥0.22 nmol/l and diabetes-related autoantibodies were randomized to receive subcutaneous injections every three months of 1 mg DiaPep277® or placebo for two years.

Importantly, the subjects treated with DiaPep277®, as in the other clinical trials, were not immuno-compromised and were spared the side effects characteristic of blanket immunosuppressive treatments. These results suggest that DiaPep277® treatment at three-monthly intervals could be continued as long as required. Additional studies will be needed to determine whether continued treatment is needed to maintain long-term treatment effects. A second, confirmatory phase III is presently underway.

5.1.6. Immune monitoring of DiaPep277® trials

Type 1 diabetes is an immune mediated disease caused by T cell-mediated destruction of the pancreatic insulin-producing beta cells. Detection of such responses is therefore critical to provide novel biomarkers for T1D 'immune staging' to understand the mechanisms underlying the disease and to monitor immune intervention studies. While different T cell assays are being developed for these purposes, it is important to optimize and standardize methods for processing human blood samples [56]. Currently there are no widely accepted and standardized assays available to analyze the function of autoreactive T cells involved in T1D [57]. Attempts are being made to validate and standardize preparation of human T cells, and to test them in proliferation assays or ELISPOT assays, tetramer analyses on CD4 positive and CD8 positive T cells are ongoing [58,59]. Immune monitoring in clinical trials in type 1 diabetes addresses several aspects: 1. the modulation of immune reactivity by treatment, 2. the natural course of the pathogenesis of type 1 diabetes and 3. safety aspects of immune interventions that could alter immune responses towards mitogens or recall antigens. Most immune intervention trials have investigated T-cell responses by proliferation assays or stimulated cytokine release. In addition, systemic immune status and cell surface analysis as measured by FACS and/or tetramers can be used. However, as none of these measurements have yet been sufficiently standardized, any results need to be interpreted with care. The heterogeneity of different assays is considerable, and it is even more complex to understand the outcome of immune monitoring from clinical trials.

Immune monitoring has been performed for most of the trials with DiaPep277®. Raz et al. reported the cytokine phenotype of the T-cell responses to HSP60 and to peptide p277 measured in vitro with a quantitative ELISpot assay [50]. In this assay, peripheral blood T cells were stimulated by incubation in vitro with the antigen and the numbers of T cells producing various cytokines were enumerated by counting spots in a cytokine capture assay. IFNγ, a Th1 cytokine, and interleukins 4, 10, and 13, Th2 cytokines, were measured. T-cell responses were also measured to bacterial recall antigens *Mycobacterium tuberculosis* (purified protein derivative; PPD) and tetanus toxoid [50]. T-cell responses to human HSP60, peptide p277, and bacterial antigens were assessed at 10 months of the trial. Patients treated by placebo showed more IFNγ and less IL-13 in response to HSP60 than to peptide p277. Thus, the whole HSP60 molecule seems

to activate more of a Th1 response than does p277 in controls. Compared with the placebo group, patients randomized to DiaPep277® treatment produced less IFNy (P = 0.04) and more IL-10 (P = 0.03) and IL-13 (P = 0.04) in response to HSP60; an increase seen in IL-4 was not significant (P = 0.14). In response to p277, DiaPep277®-treated patients produced more IL-10 (P = 0.01) and IL-13 (P = 0.02) than patients on placebo; the differences in responses to HSP60 and p277 in IL-4 and IFNy were not significant (P = 0.13 and P = 0.12, respectively). The induction of antibodies to p277 by DiaPep277® treatment could not be measured because many of the patients tested positive for such antibodies even before they received treatment [50].

Immune monitoring was also performed in the phase Ib/II trials done in Belgium, Hungary and Slovenia [60,61]. Huurman et al. [60] measured immunological efficacy of DiaPep277® treatment and correlated this with clinical outcome. T-cell autoimmunity to HSP60, DiaPep277®, glutamic acid decarboxylase and tetanus toxoid (the recall response control) from 48 patients were assayed by proliferation and cytokine secretion assays (enzyme-linked immunospot) at regular intervals until 18 months after the first injection. All treated patients at each dosage of peptide demonstrated an altered immune response to DiaPep277®, while the majority of placebo-treated patients manifested no change (P = 0.00001), indicating that an immune response was induced by DiaPep277® administration. Cytokine production in response to therapy was dominated by IL-10. IL-10 production before therapy and decreasing autoantigen-specific T cell proliferation were associated with β-cell preservation. Third-party control immune responses were unaffected by DiaPep277® therapy. No potentially adverse immunological side effects were noted [60].

Pfleger et al. applied a very similar protocol to monitor immune reactivity by ELISPOT and T-cell proliferation [61]. Fifty adults and 49 children (mean age 27.3 and 10.9 years respectively) from Slovenia and Hungary with recent onset type 1 diabetes who participated in the placebo-controlled trial with DiaPep277® were analyzed. ELISPOT assays were used to measure secretion of IFNy, IL-5, IL-13 and IL-10 by single peripheral mononuclear cells (PBMC) upon stimulation with islet antigens GAD65, HSP60, protein–tyrosine–phosphatase-like-antigen (pIA2) or tetanus toxoid (TT); β-cell function was evaluated by glucagon stimulated C-peptide. In general, DiaPep277® as well as placebo treatment were associated with decreased numbers of islet antigen-reactive cells over 78 weeks in both adults and children, whereas reactivity to the recall antigen tetanus toxoid was not reduced. In addition, there was an association between the quality of immune cell responses and β-cell function. Overall, increased responses by IFNy secreting cells were associated with lower β-cell function whereas IL-5, IL-13 and IL-10 cytokine responses were positively associated with β-cell function in adults and children. However, the clear shift towards Th2 upon DiaPep277® treatment, which had been observed in the trials reported by Raz and Huurman, was not seen [61].

In summary, immune monitoring from clinical trials with DiaPep277® has supported safety and some data show an immunological effect upon DiaPep277® treatment resulting in a shift towards Th2-related cytokine responses considered protective in the pathogenesis of type 1 diabetes. Results of immune monitoring of current phase III trials, which has been directed mainly to safety, also tend to show a stable immune status, well in line with safety [62 and manuscript in preparation NCS].

Conflicts of interest

NC Schloot has received consultant fees and scientific grants from Peptor Ltd., Develogen GmbH and Andromeda Ltd. When leading the study center at German Diabetes Center, clinical studies with DiaPep277® conducted by Peptor, Develogen and Andromeda were financially supported. Irun R. Cohen has been a consultant to all the companies involved in the development of DiaPep277® and will receive a portion of any royalties received by Yeda, the commercialization office that serves the Weizmann Institute of Science.

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References

[1] M. Rewers, R.E. LaPorte, H. King, J. Tuomilehto, Trends in the prevalence and incidence of diabetes: insulin-dependent diabetes mellitus in childhood, World Health Stat. Q. 41 (1988) 179–189.
[2] M. Rewers, J. Norris, A. Kretowski, Epidemiology of type 1 diabetes (revised 11/3/2010), chapter 9. Online textbook type 1 diabetes, Cell. Mol. Clin. Immunol. (2010)(http://barbaradaviscenter.org/).
[3] M.I. Hawa, H. Kolb, N.C. Schloot, H. Beyan, S.A. Paschou, R. Buzzetti, D. Mauricio, A. De Leiva, K. Yderstraede, H. Beck-Neilsen, J. Tuomilehto, C. Sarti, C. Thivolet, D. Hadden, S. Hunter, G. Schernthaner, W.A. Scherbaum, R. Williams, S. Brophy, P. Pozzilli, R.D. Leslie, Action LADA consortium, adult-onset autoimmune diabetes in Europe is prevalent with a broad clinical phenotype: action LADA 7, Diabetes Care 36 (2013) 908–913.
[4] EURODIAB ACE Study Group, Variation and trends in incidence of childhood diabetes in Europe, Lancet 355 (2000) 873–876.
[5] V. Harjutsalo, L. Sjoberg, J. Tuomilehto, Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study, Lancet 371 (2008) 1777–1782.
[6] S. Fourlanos, M.D. Varney, B.D. Tait, G. Morahan, M.C. Honeyman, P.G. Colman, L.C. Harrison, The rising incidence of type 1 diabetes is accounted for by cases with lower-risk human leukocyte antigen genotypes, Diabetes Care 31 (2008) 1546–1549.
[7] C.S. Zipitis, A.K. Akobeng, Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis, Arch. Dis. Child. 93 (2008) 512–517.
[8] M.W. Steffes, S. Sibley, M. Jackson, W. Thomas, Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial, Diabetes Care 26 (2003) 832–836.
[9] D. Mineo, A. Pileggi, R. Alejandro, C. Ricordi, Point: steady progress and current challenges in clinical islet transplantation, Diabetes Care 32 (2009) 1563–1569.
[10] P.J. Palmer, G.A. Fleming, C.J. Greenbaum, K.C. Herold, L.D. Jansa, H. Kolb, J.M. Lachin, K.S. Polonsky, P. Pozzilli, J.S. Skyler, M.W. Steffes, C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21–22 October 2001, Diabetes 53 (2004) 250–264.

[11] Diabetes prevention trial study grout DPT 1, Effects of insulin in relatives of patients with type 1 diabetes mellitus, N. Engl. J. Med. 346 (2002) 1685–1691.
[12] J.S. Skyler, J.P. Krischer, J. Wolfsdorf, C. Cowie, J.P. Palmer, C. Greenbaum, D. Cuthbertson, L.E. Rafkin-Mervis, H.P. Chase, E. Leschek, Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial—Type 1, Diabetes Care 28 (2005) 1068–1076.
[13] K. Näntö-Salonen, A. Kupila, S. Simell, H. Siljander, T. Salonsaari, A. Hekkala, S. Korhonen, R. Erkkola, J.I. Sipilä, L. Haavisto, M. Siltala, J. Tuominen, J. Hakalax, H. Hyöty, J. Ilonen, R. Veijola, T. Simell, M. Knip, O. Simell, 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 372 (2008) 1746–1755.
[14] J.S. Skyler, Primary and secondary prevention of type 1 diabetes, Diabet. Med. 30 (2013) 161–169.
[15] E.A. Gale, P.J. Bingley, C.L. Emmett, T. Collier, European Nicotinamide Diabetes Intervention Trial (ENDIT) Group, European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomised controlled trial of intervention before the onset of type 1 diabetes, Lancet 363 (2004) 925–931.
[16] C.J. Greenbaum, T. Mandrup-Poulsen, P.F. McGee, T. Battelino, B. Haastert, J. Ludvigsson, P. Pozzilli, J.M. Lachin, H. Kolb, Type 1 Diabetes Trial Net Research Group; European C-Peptide Trial Study Group, mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes, Diabetes Care 31 (2008) 1966–1971.
[17] A. Lernmark, H.E. Larsson, Immune therapy in type 1 diabetes mellitus, Nat. Rev. Endocrinol. 9 (2013) 92–103.
[18] L. Mastrandrea, J. Yu, T. Behrens, J. Buchlis, C. Albini, S. Fourtner, T. Quattrin, Etanercept treatment in children with new-onset type 1 diabetes: pilot randomized, placebo-controlled, double-blind study, Diabetes Care 32 (2009) 1244–1249.
[19] K.I. Rother, R.J. Brown, M.M. Morales, E. Wright, Z. Duan, C. Campbell, D.S. Hardin, J. Popovic, R.C. McEvoy, D.M. Harlan, P.R. Orlander, S.A. Brod, Effect of ingested interferon-alpha on beta-cell function in children with new-onset type 1 diabetes, Diabetes Care 32 (2009) 1250–1255.
[20] S. Martin, C. Herder, N.C. Schloot, W. Koenig, T. Heise, L. Heinemann, H. Kolb, DIATOR Study Group. Residual beta cell function in newly diagnosed type 1 diabetes after treatment with atorvastatin: the Randomized DIATOR Trial, PLoS One 11 (2011) e17554.
[21] A.S. Wierzbicki, Atorvastatin, Expert. Opin. Pharmacother. 5 (2001) 819–830.
[22] A. Strom, H. Kolb, S. Martin, C. Herder, M.C. Simon, W. Koenig, T. Heise, L. Heinemann, M. Roden, N.C. Schloot, DIATOR Study Group, Improved preservation of residual beta cell function by atorvastatin in patients with recent onset type 1 diabetes and high CRP levels (DIATOR trial), PLoS One 7 (2012) e33108.
[23] C.M. Larsen, M. Faulenbach, A. Vaag, A. Vølund, J.A. Ehses, B. Seifert, T. Mandrup-Poulsen, M.Y. Donath, Interleukin-1-receptor antagonist in type 2 diabetes mellitus, N. Engl. J. Med. 356 (2007) 1517–1526.
[24] C. Pfleger, H.B. Mortensen, L. Hansen, C. Herder, B.O. Roep, H. Hoey, H.J. Aanstoot, M. Kocova, N.C. Schloot, Hvidøre Study Group on Childhood Diabetes, Association of IL-1ra and adiponectin with C-peptide and remission in patients with type 1 diabetes, Diabetes 57 (2008) 929–937.
[25] L.M. Pickersgill, T. Mandrup-Poulsen, On behalf of the AIDA study group, the anti-interleukin-1 in type 1 diabetes action trial—background and rationale, Diabetes Metab. Res. Rev. 25 (2009) 321–324.
[26] A. Moran, B. Bundy, D.J. Becker, L.A. DiMeglio, S.E. Gitelman, R. Goland, C.J. Greenbaum, K.C. Herold, J.B. Marks, P. Raskin, S. Sanda, D. Schatz, D.K. Wherrett, D.M. Wilson, J.P. Krischer, J.S. Skyler*, Type 1 Diabetes TrialNet Canakinumab Study Group**, L. Pickersgill, E. de Koning, A.-G. Ziegler, B. Böhm, K. Badenhoop, N.C. Schloot, J.F. Bak, P. Pozzilli, D. Mauricio, M.Y. Donath, L. Castaño, A. Wägner, H.H. Lervang, H. Perrild, T. Mandrup-Poulsen*, On behalf of the AIDA Study Group Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicenter, randomized double-masked, placebo-controlled trials, Lancet 381 (2013) 1905–1915.
[27] B. Keymeulen, E. Vandemeulebroucke, A.G. Ziegler, C. Mathieu, L. Kaufman, G. Hale, F. Gorus, M. Goldman, M. Walter, S. Candon, L. Schandene, L. Crenier, C. De Block, J.M. Seigneurin, P. De Pauw, D. Pierard, I. Weets, P. Rebello, P. Bird, E. Berrie, M. Frewin, H. Waldmann, J.F. Bach, D. Pipeleers, L. Chatenoud, Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes, N. Engl. J. Med. 352 (2005) 2598–2608.
[28] K.C. Herold, S.E. Gitelman, U. Masharani, W. Hagopian, B. Bisikirska, D. Donaldson, K. Rother, B. Diamond, D.M. Harlan, J.A. Bluestone, A single course of anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes, Diabetes 54 (2005) 1763–1769.
[29] N. Sherry, W. Hagopian, J. Ludvigsson, S.M. Jain, J. Wahlen, R.J. Ferry Jr., B. Bode, S. Aronoff, C. Holland, D. Carlin, K.L. King, R.L. Wilder, S. Pillemer, E. Bonvini, S. Johnson, K.E. Stein, S. Koenig, K.C. Herold, A.G. Daifotis, Protégé Trial Investigators, Teplizumab for treatment of type 1 diabetes (Protégé study): 1-year results from a randomised, placebo-controlled trial, Lancet 378 (2011) 487–497.
[30] J. Lebastchi, S. Deng, A.H. Lebastchi, I. Beshar, S. Gitelman, S. Willi, P. Gottlieb, E.M. Akirav, J.A. Bluestone, K.C. Herold, Immune therapy and β-cell death in type 1 diabetes, Diabetes 62 (2013) 1676–1680.
[31] S. Martin, D. Wolf-Eichbaum, G. Duinkerken, W.A. Scherbaum, H. Kolb, J.G. Noordzij, B.O. Roep, Development of type 1 diabetes despite severe hereditary B-lymphocyte deficiency, N. Engl. J. Med. 345 (2001) 1036–1040.
[32] M.D. Pescovitz, C.J. Greenbaum, H. Krause-Steinrauf, D.J. Becker, S.E. Gitelman, R. Goland, P.A. Gottlieb, J.B. Marks, P.F. McGee, A.M. Moran, P. Raskin, H. Rodriguez, D.A. Schatz, D. Wherrett, D.M. Wilson, J.M. Lachin, J.S. Skyler, Type 1 Diabetes TrialNet Anti-CD20 Study Group, Rituximab, B-lymphocyte depletion, and preservation of beta-cell function, N. Engl. J. Med. 361 (2009) 2143–2152.
[33] J. Ludvigsson, M. Faresjö, M. Hjorth, S. Axelsson, M. Chéramy, M. Pihl, O. Vaarala, G. Forsander, S. Ivarsson, C. Johansson, A. Lindh, N.O. Nilsson, J. Aman, E. Ortqvist, P. Zerhouni, R. Casas, GAD treatment and insulin secretion in recent-onset type 1diabetes, N. Engl. J. Med. 359 (2008) 1909–1920.
[34] J. Ludvigsson, D. Krisky, R. Casas, T. Battelino, L. Castaño, J. Greening, O. Kordonouri, T. Otonkoski, P. Pozzilli, J.J. Robert, H.J. Veeze, J. Palmer, GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus, N. Engl. J. Med. 366 (2012) 433–442.
[35] B.O. Roep, N. Solvason, P.A. Gottlieb, J.R. Abreu, L.C. Harrison, G.S. Eisenbarth, L. Yu, M. Leviten, W.A. Hagopian, J.B. Buse, M. von Herrath, J. Quan, R.S. King, W.H. Robinson, P.J. Utz, H. Garren, BHT-3021 Investigators, Steinman L, plasmid-encoded proinsulin preserves C-peptide while specifically reducing proinsulin-specific CD8+ T cells in type 1 diabetes, Sci. Transl. Med. 5 (2013) 191ra82.
[36] D. Elias, T. Reshef, O.S. Birk, R. van der Zee, M.D. Walker, I.R. Cohen, 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. 88 (1991) 3088–3091.
[37] D. Elias, I.R. Cohen, Peptide therapy for diabetes in NOD mice, Lancet 343 (1994) 704–706.
[38] F.J. Quintana, I.R. Cohen, The HSP60 immune system network, Trends Immunol. 32 (2011) 89–95.

[39] S.H. Kaufmann, Heat shock proteins and the immune response, Immunol. Today 11 (1990) 129–136.
[40] Y. Merbl, M. Zucker-Toledano, F.J. Quintana, I.R. Cohen, Newborn humans manifest autoantibodies to defined self molecules detected by antigen microarray informatics, J. Clin. Invest. 117 (2007) 712–718.
[41] F.J. Quintana, P.H. Hagedorn, G. Elizur, Y. Merbl, E. Domany, I.R. Cohen, Functional immunomics: microarray analysis of IgG autoantibody repertoires predicts the future response of mice to induced diabetes, Proc. Natl. Acad. Sci. U. S. A. 5 (101 Suppl. 2) (2004) 14615–14621.
[42] F.J. Quintana, A. Rotem, P. Carmi, I.R. Cohen, Vaccination with empty plasmid DNA or CpG oligonucleotide inhibits diabetes in nonobese diabetic mice: modulation of spontaneous 60-kDa heat shock protein autoimmunity, J. Immunol. 165 (2000) 6148–6155.
[43] D. Elias, I.R. Cohen, The hsp60 peptide p277 arrests the autoimmune diabetes induced by the toxin streptozotocin, Diabetes 45 (1996) 1168–1172.
[44] V. Ablamunits, D. Elias, T. Reshef, I.R. Cohen, Islet T cells secreting IFN-gamma in NOD mouse diabetes: arrest by p277 peptide treatment, J. Autoimmun. 11 (1998) 73–81.
[45] D. Elias, I.R. Cohen, Treatment of autoimmune diabetes and insulitis in NOD mice with heat shock protein 60 peptide p277, Diabetes 44 (1995) 1132–1138.
[46] I. Raz, D. Elias, A. Avron, M. Tamir, M. Metzger, I.R. Cohen, 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 358 (2001) 1749–1753.
[47] A. Zanin-Zhorov, G. Tal, S. Shivtiel, M. Cohen, T. Lapidot, G. Nussbaum, R. Margalit, I.R. Cohen, O, Heat shock protein 60 activates cytokine-associated negative regulator suppressor of cytokine signaling 3 in T cells: effects on signaling, chemotaxis, and inflammation, J. Immunol. 175 (2005) 276–285.
[48] A. Zanin-Zhorov, G. Nussbaum, S. Franitza, I.R. Cohen, O. Lider, T cells respond to heat shock protein 60 via TLR2: activation of adhesion and inhibition of chemokine receptors, FASEB J. 17 (2003) 1567–1569.
[49] D. Tuccinardi, E. Fioriti, S. Manfrini, E. D'Amico, P. Pozzilli, DiaPep277® peptide therapy in the context of other immune intervention trials in type 1 diabetes, Expert. Opin. Biol. Ther. 11 (2011) 1233–1240.
[50] I. Raz, A. Avron, M. Tamir, M. Metzger, L. Symer, R. Eldor, I.R. Cohen, D. Elias, 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. 23 (2007) 292–298.
[51] V.A. Huurman, P. van der Meide, G. Duinkerken, S. Willemen, I.R. Cohen IR, D. Elias, B.O. Roep, Immunological efficacy of heat shock protein 60 peptide DiaPep277® therapy in clinical type I diabetes, Clin. Exp. Immunol. 152 (2008) 488–497.
[52] N.C. Schloot, G. Meierhoff, C. Lengyel, G. Vandorfi, J. Takacs, P. Panczel, L. Barkai, L. Madacsy, T. Oroszlan, P. Kovacs, G. Suto, T. Battelino, N. Hosszufalusi, G. Jermendy, 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. 23 (2007) 276–285.
[53] R. Buzzetti, S. Cernea, A. Petrone, M. Capizzi, M. Spoletini, S. Zampetti, C. Guglielmi, C. Venditti, P. Pozzilli, DiaPep Trialists Group. C-peptide response and HLA genotypes in subjects with recent-onset type 1 diabetes after immunotherapy with DiaPep277®: an exploratory study, Diabetes 60 (2011) 3067–3072.
[54] P. Pozzilli, C. Guglielmi, Immunomodulation for the prevention of SPIDDM and LADA, Ann. N. Y. Acad. Sci. 1079 (2006) 90–98.
[55] L. Lazar, R. Ofan, N. Weintrob, A. Avron, M. Tamir, D. Elias, M. Phillip, Z. Josefsberg, 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. 23 (2007) 286–291.
[56] R. Mallone, S.I. Mannering, B.M. Brooks-Worrell, I. Durinovic-Belló, C.M. Cilio, F.S. Wong, N.C. Schloot, T-Cell Workshop Committee, Immunology of Diabetes Society, Isolation and preservation of peripheral blood mononuclear cells for analysis of islet antigen-reactive T cell responses: position statement of the T-Cell Workshop Committee of the Immunology of Diabetes Society, Clin. Exp. Immunol. 163 (2011) 33–49.
[57] S.I. Mannering, F.S. Wong, I. Durinovic-Belló, B. Brooks-Worrell, T. Tree, C.M. Cilio, N.C. Schloot, R. Mallone, Immunology of Diabetes Society T-Cell Workshop Committee. Current approaches to measuring human islet-antigen specific T cell function in type 1 diabetes, Clin. Exp. Immunol. 162 (2010) 197–209.
[58] E.A. James, R. Mallone, N.C. Schloot, M.C. Gagnerault, J. Thorpe, L. Fitzgerald-Miller, J. Reichow, R. Wagner, M.N. Pham, N. Jospe, O. Lou, P.A. Gottlieb, B. Brooks-Worrell, I. Durinovic-Belló, T-Cell Workshop Committee, Immunology of Diabetes Society, Immunology of Diabetes Society T-Cell Workshop: HLA class II tetramer-directed epitope validation initiative, Diabetes Metab. Res. Rev. 27 (2011) 727–736.
[59] B. Brooks-Worrell, T. Tree, S.I. Mannering, I. Durinovic-Bello, E. James, P. Gottlieb, S. Wong, Z. Zhou, L. Yang, C.M. Cilio, J. Reichow, B. Menart, R. Rutter, R. Schreiner, M.N. Pham, L. Petrich de Marquesini, O. Lou, M. Scotto, R. Mallone, N.C. Schloot N, T-Cell Workshop Committee, Immunology of Diabetes Society, Comparison of cryopreservation methods on T-cell responses to islet and control antigens from type 1 diabetic patients and controls, Diabetes Metab. Res. Rev. 27 (2011) 737–745.
[60] V.A. Huurman, K. Decochez, C. Mathieu, I.R. Cohen, B.O. Roep, Therapy with the hsp60 peptide DiaPep277® in C-peptide positive type 1 diabetes patients, Diabetes Metab. Res. Rev. 23 (2007) 269–275.
[61] C. Pfleger, G. Meierhoff, H. Kolb, N.C. Schloot, p520/521 Study Group, association of T-cell reactivity with beta-cell function in recent onset type 1 diabetes patients, J. Autoimmun. 34 (2010) 127–135.
[62] S. Dagan, A.G. Ziegler, P.G. Pozzilli, T. Linn, G. Schernthaner, F. Bonnici, D. Elias, R. Eren, I.R. Cohen, I. Raz, S. Group, Recent Data from DIA-AID 1, a Global Phase III Clinical Study in Newly Diagnosed Type 1 Diabetes Patients, in Diabetes, ADA 292-OR Oral Presentation, 2012. (Philadelphia, USA).