Published in final edited form as:

J Intern Med. 2011 June ; 269(6): 626–635. doi:10.1111/j.1365-2796.2011.02386.x.

Vaccination against type 1 diabetes

Helena Elding Larsson and Åke Lernmark

Department of Clinical Sciences, Lund University/CRC, Skåne University Hospital SUS, SE-20502 Malmö, Sweden

Åke Lernmark: Ake.Lernmark@med.lu.se

Abstract

The clinical onset of type 1 diabetes or autoimmune diabetes occurs after a prodrome of islet autoimmunity. The warning signals for the ensuing loss of pancreatic islet beta cells are autoantibodies against insulin, GAD65, IA-2, and ZnT8, alone or in combinations. Autoantibodies against e.g. insulin alone have only a minor risk for type 1 diabetes. However, progression to clinical onset is increased by the induction of multiple islet autoantibodies. At the time of clinical onset, insulinitis may be manifest, which seem to reduce the efficacy of immunosuppression. Autoantigen-specific immunotherapy with alum-formulated GAD65 (Diamyd®) show promise to reduce the loss of beta-cell function after the clinical onset of type 1 diabetes. The mechanisms are unclear but may involve the induction of T regulatory cells, which may suppress islet autoantigen reactivity. Past and on-going clinical trials have been safe. Future clinical trials, perhaps as combination autoantigen-specific immunotherapy may increase the efficacy to prevent the clinical onset in subjects with islet autoantibodies or preserve residual beta-cell function in newly diagnosed type 1 diabetes patients.

Introduction

Type 1 or autoimmune diabetes is a remarkable disorder that exemplifies the unsurpassed ability of the immune system to recognize and destroy a target in a highly specific manner. The pancreatic islet beta cells are the target in islet autoimmunity. While islet autoimmunity is a symptomless and subclinical condition, type 1 diabetes is the end of a prodrome that may have been present for years [1, 2]. Leaving the neighboring cells producing glucagon, somatostatin and pancreatic polypeptide untouched, the destruction of the islet beta cells eventually results in a complete lack of insulin production[3]. However, the clinical onset of type 1 diabetes varies with age and unpredictable amount of residual beta-cell function [4–6]. Children younger than five years of age have less residual beta-cell function compared to teen-agers or young adults [4, 7]. In adult autoimmune diabetes, often referred to Latent Autoimmune Diabetes in Adults (LADA) (reviewed in [8, 9], residual beta-cell function is often within the normal range at the time of clinical diagnosis [10]. The consequence of the autoimmune attack is that the patient is left to be dependent on lifelong daily insulin injections. Spontaneous remissions are not heard of.

The many approaches to insulin replacement therapy and control of blood glucose are steadily improving. However, insulin treatment is not a cure for type 1 diabetes. It is nothing but a replacement therapy of a hormone that is no longer produced because the beta cells are destroyed. Significant resources are spent by the industry to develop new insulin formulations and insulin analogues. Better needles and insulin pumps, pens and other

Conflict of interest

ÅL is a member of the Scientific Advisory Board of Diamyd Medical AB, Stockholm, Sweden.

devices, blood glucose meters, continuous glucose monitors and emerging technologies to combine pumps with meters to an artificial pancreas [11]. Clinical islet and pancreas transplantation has only made limited progress during almost 40 years of effort [12, 13]. Recurrence of the disease is common [14], which complicates the efforts to prevent allograft rejection by standard immune suppressive regimens [13]. It is remarkable that the underlying islet autoimmune disease is still able to overcome the advanced immunosuppression used to treat type 1 diabetes patients receiving islets [12–14]. Taken together, unless the underlying disease process – the beta cell autoantigen-specific immune pathogenesis is reversed or inhibited there seem to be little hope for clinical islet transplantation to be successful.

Should autoimmune diabetes be treated with immunosuppressive drugs?

The very logic from the recognition in the early 1970ies that type 1 diabetes is an autoimmune disease was that the immune attack ought to be met by immunosuppression. The very first report was by late David A. Pyke who treated islet cell antibody positive patients with intravenous prednisolone (reviewed in [15]). This initial attempt to preserve beta-cell function by immunosuppression was soon to be followed by numerous case-reports or open-labeled, uncontrolled clinical studies using various immunosuppressive agents, as they become available. The results overall were negative. It was early questioned that the use of immunosuppressive strategies to impede or halt complete destruction of beta cells was inappropriate because of potential toxicity of all current immunosuppressive regimens [16]. The number of newly diagnosed type 1 diabetes patients who had been treated with different immunosuppressive agents was large already in 1987 [17] and has continued to increase [18]. Initially immunosuppressive agents were used in open labeled, non-randomized, uncontrolled trials [17]. Indeed, the first multicenter, placebo controlled random clinical trials were with cyclosporine [19, 20]. Although residual beta-cell function was transiently preserved, cyclosporine had to be abandoned due to lack of long-term effects and nephrotoxicity [21]. The approach to immunosuppression was revived with the advent of humanized monoclonal antibody biologicals. In particular, the use of anti-CD3 (three different brands are currently evaluated in controlled clinical trials) has been in the forefront of efforts [18]. Current data indicate that anti-CD3 treatment preserve beta-cell function in a transient manner [18]. It is still unclear what the patient benefits are in a relation to a transient preservation of beta-cell function and the long term risk of treatment with immunosuppressive agents [22]. Recently, treatment with Rituximab, a CD20 monoclonal antibody showed preservation of beta-cell function after the clinical diagnosis that as good as treatment with anti-CD3 [5]. The results with Rituximab underscore the importance of B-lymphocytes as possible antigen-presenting cells in the pathogenesis of islet autoimmunity and type 1 diabetes. Indeed, B-lymphocytes as antigen-presenting cells may be responsible for the chronic anti-islet autoantigen response as exemplified in in vitro experiments with human B-lymphocytes presenting GAD65 on HLA Class II antigens [23, 24]. Taken together, immunosuppressive agents seem do not seem to be able to block the autoimmune process that is associated with loss of the pancreatic islet beta cells. Effects are transient. The lack of reproducible, long-lasting effects of immunosuppressive treatment at the time of clinical diagnosis of type 1 diabetes has made it necessary to explore alternative approaches to preserve the residual beta-cell function at the time of clinical diagnosis. Both non-antigen specific (NASI) as well as antigen-specific immunotherapy (ASI) have been attempted. These approaches to treatment, provided they are safe also include studies of primary prevention, secondary prevention in subjects with persistent islet autoimmunity as well as intervention at the time of clinical diagnosis of type 1 diabetes.

The underlying problem

The underlying problem is a remarkable ability of the human immune system to mount highly specific immune responses not only to specific antigens but also to unique epitopes of the antigen. In addition to the exquisite specificity, there is also memory built into the immune reaction. This is best exemplified by vaccination. Vaccinating an infant with attenuated virus is both specific and life long. The vaccination is assumed to mimic a natural infection. It involves mechanisms of antigen-uptake i.e. uptake of attenuated virus by antigen-presenting cells (APC). The virus proteins are subjected to proteolysis and the resulting virus peptide fragments are loaded onto HLA class II heterodimeric proteins. The trimolecular complex thus formed is displayed on the cell surface of the APC to be seen by T cell receptors on CD4+ T cells that are passing by. The TCR is highly specific to recognize the HLA class II specificity of the individual along with the peptide of the processed antigen. The contact between the APC and the TCR on the CD4+ T helper cell is key to the subsequent cascade of events, which eventually will result in the generation of both CD8+ T cells - able to attack virus-infected cells – and of B lymphocytes - able to produce antibodies against virus antigen (Figure 1). Cytokines produced by the T cells influence whether the immune response will be dominated by either cellular or humoral reactions. It is an oversimplification that cellular reactivity takes place without humoral reactivity, and vice versa. Cytokines such as IFN γ and IL-2 tend to promote cellular (referred to as a Th1 response) while IL-4 and IL-10 promote humoral (referred to as a Th2 responses) reactivity.

Both arms of the immune response are required to effectively neutralize a virus infection. The cellular arm seems not to be independent of the humoral as B cells are highly effective APC. Clearing a virus infection is strongly associated with virus antibody titers. Adjuvant is used in all vaccines not only to achieve high titer responses but also secure a shift of the immune reaction to a humoral response. The immune response developed to either a live virus or to an attenuated virus is life long through memory T and B cells [25]. This is at the heart of the problem in type 1 diabetes. The autoimmune response occurs against self-antigens, often intra cellular, membrane-associated proteins, such as GAD65, IA-2 and the ZnT8 transporter protein [26, 27]. It is thought that APC processing and presentation of autoantigens take place with the same mechanisms as for exogenous antigens. There is a major lack in knowledge as to the detailed mechanisms and what the trigger(s) might be of the autoimmune response. The bottom line is, however, undisputable: the immune response induced against the beta-cell autoantigens GAD65, insulin, IA-2 or ZnT8 are life-long. Also, it is reasonable to assume that beta-cell autoantigens are lost in parallel with the loss of beta cells. Hence, when an islet autoantigen is decreasing there will also be a decrease in the corresponding islet autoantibody. We speculate that autoantibody-levels against an islet autoantigen may decrease in proportion to remaining beta cells. Taken together, the underlying problem is a chronic autoimmune response against specific epitopes on islet autoantigens. The autoimmune response seems established and epitope-specificity does not seem to be modulated by ASI [28, 29]. Memory T and B-lymphocytes secure that the autoantigen is remembered for life. Intuitively, immunosuppression would seem to be almost contraindicated as most of immunosuppressive agents interfere with early events in the immune response. Future treatment of islet autoimmunity may have to focus on immune tolerance induction as soon as memory T and B-lymphocytes against islet autoantigens have been established.

Immunological tolerance

The human immune response includes mechanisms that protect the individual from destroying itself by autoimmunity. The first key event is elimination in the thymus of T cells

expressing TCR that recognize self-peptides. The second key event is death in the periphery of self-reacting T cells. Self-peptides are continuously presented on APC. Low-level autoantigen presentation is thought to induce apoptosis in self-reactive T cells. Elimination of self-reactive T cells is central to maintaining immunological tolerance. CD4⁺ T cells with TCR recognizing a self-antigen needs to go into apoptosis to be eliminated. Numerous hypotheses have been developed to explain mechanisms of break or loss of tolerance. HLA class II molecules may be critical to establish and maintain immunological tolerance. It cannot be excluded that the two major HLA-DQ haplotypes (DQ A1*05:01-B1*02:01 (DQ2) and DQ A1*03:01-B1*03:02 (DQ8), which are associated with type 1 diabetes may be less effective in preventing autoimmunity. It is speculated that the induction and maintenance of immunological tolerance may work well for some but not all HLA heterodimers. Furthermore, dendritic cells (DC) are critical to the immune system because they are both effective antigen-presenting cells in inducing adaptive immune responses but also critically involved in promoting and maintaining immunological tolerance. The latter function is mediated by tolerogenic DC, a specialized subset of DC as well as by DC activated or differentiated for example by autoantigens. Tolerogenic DC seems to control suppression primarily via the induction of regulatory T (Treg) cells.

Triggering the beta cell autoimmune response

The incidence of type 1 diabetes is highest in Scandinavia (30–50/100,000), intermediate in the United States (15–25/100,000 in 1998), and somewhat lower in Central and Eastern Europe (5–15/100,000) [30]. It is unclear to what extent these geographic differences may reflect variation in genetic susceptibility, in prevalence of causal environmental factors, or both. The dissection of the etiology of type 1 diabetes is further complicated by epidemiological observations that approximately 85–90% of new onset type 1 diabetes patients do not have a first degree relative (FDR) with the disease [30]. Genetic variability in the HLA region is thought to explain about 50% of the familial clustering but other genes identified so far provide more modest contributions to risk [31]. As indicated above, the mechanisms by which environmental factors may trigger beta-cell autoimmunity in susceptible subjects are not understood. Epidemiological studies have associated type 1 diabetes with gestational infections or with infections early in life [32]. Development of standardized islet autoantibody tests [33, 34] has made it possible to initiate studies to follow children with increased genetic risk for type 1 diabetes from birth to record the appearance of islet autoantibodies [35–39]. Most of these studies may lack statistical power to identify a casual environmental trigger; their recent reports demonstrate early seroconversion and prolonged subclinical autoantibody positivity prior to clinical onset of type 1 diabetes [36]. Indeed, the prodrome may be associated with islet autoantibodies that may come and go before hyperglycemia. The number of islet autoantibodies experienced, not necessarily the number of autoantibodies at the time of diagnosis appears to determine the time to clinical onset [1, 36]. Understanding the trigger(s) of autoantibodies against insulin, GAD65, IA-2 or ZnT8 will be critical to future attempts to modulate the islet autoreactivity as early as possible as to avoid a chronic autoreactivity to islet autoantigens. The possibility to interfere with autoantibody formation against islet autoantigens may be the most efficient way to prevent type 1 diabetes. Formation of beta cell autoantibodies signifies a break of tolerance. Would immune tolerance induction be more effective in the early beta-cell autoantibody positive individual compared to patients who present with chronic beta cell autoimmunity at the time of clinical diagnosis?

The chronic beta cell autoimmune response

During the period preceding the clinical onset, autoantibodies targeting specific islet autoantigens such as insulin, glutamic acid decarboxylase (GAD65), islet antigen-2 (IA-2)

and zinc transporter (ZnT8) may be detectable for months up to years before hyperglycemia [2, 35]. The clinical diagnosis of type 1 diabetes is undisputedly associated with a major loss of beta cells [3, 40]. Insulinitis, viewed by many to be the hallmark of type 1 diabetes pathology may neither be detected in all newly diagnosed type 1 diabetes patients [3] nor in subjects positive for only one islet autoantibody [40]. In contrast to dogma, insulinitis i.e. infiltration of the islet of Langerhans by mononuclear cells may not represent a pathological phenomenon that explains the appearance of islet autoantibodies. Rather, when several islet autoantibodies are present insulinitis may develop as the final reaction that is accelerating beta-cell loss prior to the clinical onset of type 1 diabetes [40]. A recent study detected islet autoantibodies among 62 (4%) individuals out of 1507 pancreatic donors aged 25–60 years [40]. Although those 62 individuals also had HLA susceptibility only two of them showed insulinitis, indicating that the presence of islet autoantibodies was not necessarily a marker of insulinitis. The exact role of the autoantibody warning signals are therefore not understood and it needs to be established to what extent they are markers of beta-cell destruction by cellular autoimmunity. Insulinitis seems rather detected late in chronic beta cell autoimmunity, indeed close to the clinical onset of the disease. The chronic beta cell autoimmunity may therefore be characterized of an early triggering mechanism that builds up over time.

The mechanism involved in beta-cell destruction is not yet fully clear. One possible scenario is that beta cells are first destroyed by an environmental factor such as a virus. The dying or dead beta cell is next phagocytosed by local dendritic cells (DC). The DC is activated and migrates through the lymphatics to the lymph nodes draining the pancreas. The autoantigen presentation to and activation of CD4⁺ T lymphocytes would take place in the lymph node to include activation of CD8⁺ T lymphocytes specific for islet autoantigen and perhaps also of B-lymphocytes that would produce autoantibodies against either insulin, GAD65, IA-2 or ZnT8. The islet autoantigen-specific CD8⁺ T lymphocytes are thought to be able to return to the blood circulation. These killer cells may eventually end up in an islet to begin the destruction of additional beta cells. The beta-cell killing will generate a new cycle of DC and B-lymphocyte mediated islet autoantigen presentations in the draining lymph nodes of the pancreas resulting in the recruitment of T and B lymphocytes recognizing another epitope on the initial autoantigen or perhaps additional autoantigens. This phenomenon is known as epitope spreading [41–43]. CD4⁺CD25⁺ Treg lymphocytes may be important to as they may inhibit islet autoantigen specific CD4⁺ T lymphocytes [54]. These cells express FOXP3 from the X chromosome and are important to maintain peripheral tolerance.

GAD65-specific immunotherapy – early animal studies

GAD65 is a major autoantigen in type 1 diabetes. More than 65 % of type 1 diabetes patients have GAD65 autoantibodies against GAD65 at the time of clinical onset of type 1 diabetes [10, 26]. GAD65 autoantibodies may disappear before the clinical onset; GAD65 autoantibodies may have been present in an even higher proportion of the patients who eventually develop type 1 diabetes. Molecular cloning of human islet GAD65 identified the novel isoform of GAD [44]. When recombinant GAD65 was injected intraperitoneally into the spontaneously non-obese diabetic (NOD) mouse, the onset of diabetes was found to be delayed compared to control mice [45, 46]. Later GAD65 was found to modulate the immune response and induce T-cell tolerance as well as inhibit disease progression in NOD mice [47]. Another study showed that the inhibition of disease progression was mediated through induction of GAD65-specific regulatory T cells [48]. Less pancreatic inflammation was found in GAD65-treated NOD mice compared to controls. More important, when ordinary mice, not prone to diabetes, were treated with GAD65, no inflammation around the pancreatic islets was found, suggesting that immunization with GAD65 do not induce beta-cell autoimmunity or diabetes [49]. These animal findings suggested that treatment with

recombinant human GAD65 might be useful to modulate the GAD65 autoreactivity in type 1 diabetes.

GAD65-specific immunotherapy – early human studies

Skin prick tests were carried out in seven young adults with type 1 diabetes and in eight healthy controls to test whether type 1 diabetes was associated by immunological sensitization to recombinant human GAD65. All but one of the type 1 diabetes patients but none of the controls had GAD65 autoantibodies. The skin prick test to GAD65 was negative in all subjects. All control substances including the autoantigen vehicle, human serum albumin, PPD and candida albicans were also negative. This study did not reveal that patients with type 1 diabetes of short duration have skin prick test reactivity to recombinant human GAD65.

Diamyd Medical AB (<http://www.diamyd.com/>) carried out a Phase I study in the UK by treating 16 non-DR3-DQ2 and non-DR4-DQ8, healthy Caucasian male volunteers with a single subcutaneous injection of unformulated recombinant human GAD65. This rising, single-dose, double blind study, which also included eight volunteers receiving placebo, was conducted to assess the safety and tolerability after subcutaneous administration. The dose levels were ascending from 20 to a maximum of 500 microgram of unformulated recombinant human GAD65 per volunteer. No significant treatment-related adverse clinical effects were seen at any dose-level. GAD65, insulin, or IA-2 autoantibodies were not induced in any subject. Accordingly, it was concluded that this GAD65 treatment was clinically safe and well tolerated.

A Phase II trial in 47 patients with Latent Autoimmune Diabetes in Adults (LADA) was carried out as a randomised, placebo-controlled, dose-escalating study [29, 50–52]. Placebo or 4, 20, 100 and 500 microgram alum-formulated GAD65 was given subcutaneously week one and four. Both fasting and stimulated C-peptide levels were increased in LADA patients who received 20 microgram. No study-related serious adverse events were reported during the first 24 months follow-up [51]. After 5 years, fasting C-peptide levels declined in the placebo and the 500 microgram dose groups, respectively [52]. Some of the patients in the 100-microgram dose-groups had a reduced loss of residual c-peptide levels. No severe study-related adverse events occurred and the primary outcome of safety was achieved.

Mechanistic studies suggested that CD4+CD25+ T cells were increased in the 20-microgram-test group[51]. Analyses of the GAD65 autoantibody epitope pattern revealed only minor changes with 4, 20 or 100 microgram GAD65. In contrast, in the 500-microgram-dose group there was an increase in GAD65 autoantibody levels to a conformational epitope located in the middle part of GAD65. GAD65 autoantibodies reactive with this epitope are strongly associated with type 1 diabetes risk [28, 29]. A recent observation suggests that anti-idiotypic antibodies (anti-Id) against GAD65 autoantibodies specific to the middle epitope of GAD65 are decreased or non-detectable in children with newly diagnosed type 1 diabetes [53]. Following the vaccination with 20 microgram alum-formulated GAD65, it was observed that anti-Id levels declined in seven of nine patients of the LADA patients receiving placebo, whereas four of five patients receiving 20 microgram alum-formulated GAD65 showed increasing anti-Id levels [54]. These results are consistent with the possibility that low level alum-formulated GAD65 has a tolerogenic effect on GAD65 autoreactivity.

Diamyd Medical AB next carried out a Phase IIb clinical trial (NCT00435981) in children 10–18 years of age, with recent onset of type 1 diabetes [6, 55, 56]. A total of 70 children were included in this placebo-controlled, double-blind study where 20 microgram alum-formulated GAD65 was given day one and 30. Overall the study provided strong support for

the clinical safety of the drug [6, 55, 56]. The pancreatic beta cell function, measured as fasting and stimulated C-peptide, was significantly preserved [6]. The data further suggested that alum-formulated GAD65 may be most effective in children with disease duration less than three months [6, 56].

Mechanistic studies suggested that alum-formulated GAD65 reduced IgG1 and induced IgG3 and IgG4 GAD65 autoantibodies suggesting a Th2 deviation of the immune response. It was also observed that higher GAD65 autoantibody titers at entry were associated with better c-peptide preservation [57]. Ex vivo analysis of T cells further suggested that treatment with 20 microgram alum-formulated GAD65 might have induced T cells with regulatory features [58].

Antigen-specific immunotherapy – primary prevention trials

A first primary intervention trial, Pre-POINT has been initiated to use insulin in infants at high genetic risk (HLA-DQ2/8 heterozygous children). This trial of children born in families with type 1 diabetes (mother, father or sibling also known as FDR) attempts at identifying the dose and the type of insulin that may prevent progression to insulin autoantibody formation and subsequent progression to type 1 diabetes. The Pre-POINT is an ongoing trial, which has two arms, oral and intranasal arms. This international effort recruits genetically predisposed FDR infants aged 18 months to 7 years with no islet autoimmunity. In this trial, oral insulin dose is almost 10 times higher than that used in the DPT-1 trial (<http://www.diabetes-point.org/nav2uk.html>).

Antigen-specific immunotherapy – secondary prevention trials

Insulin

Secondary prevention trials are mainly intended for genetically predisposed children and young adults with multiple islet cell autoantibodies. These trials aim at reducing the islet autoimmunity and preventing progression to clinical diabetes [1, 37, 59]. In the DPT-1 study, it was possible to identify individuals with islet cell autoantibodies in families with type 1 diabetes. These subjects were enrolled into a large, multicenter, randomized, controlled clinical trial with either parenteral [1] or oral [60] insulin. Neither treatment delayed or prevented type 1 diabetes. A subgroup of subjects with high titer insulin autoantibodies seemed to have a delayed clinical onset of type 1 diabetes [60]. Further studies of orally administered insulin in subjects with high titers of insulin autoantibodies are ongoing (NCT00419562). The DIPP study in Finland was a double-blind trial, which used nasal insulin in children with genetic risk and positive insulin autoantibodies. In 224 children short acting insulin or placebo was administered intranasally once a day, but no protective effect was seen [37]. Intranasal Insulin Trial (INIT) in Australia (NCT00336674) is an ongoing randomized; double blind, placebo-controlled trial using nasal insulin (1.6 mg or 16 mg). The aims are also to assess effects of nasal insulin on islet autoimmunity as well as progression to clinical onset. None of the above studies have shown adverse events.

GAD65

The phase IIb study indicated that alum-formulated GAD65 may better reserve beta-cell function in children with short disease duration [6]. No study-related adverse events were reported in the Phase IIb study. It would therefore be rational to intervene even earlier, when more functioning beta cells still remain, ideally during the prodrome of islet autoimmunity perhaps long before the clinical onset. The purpose of this double blind, randomized investigator-initiated study (NCT01122446) is to determine the safety and the effect of alum-formulated GAD65 on the progression to type 1 diabetes in children with multiple islet cell autoantibodies included GAD65 autoantibodies. Eligible children are 4 years or older

but younger than 17.99 years of age, have positive GAD65 autoantibodies and at least one additional autoantibody and not yet diabetes. The study will in total include 50 children. Half of the participants (n=25) will have two injections of 20 microgram alum-formulated GAD65 on day one and 30 and half (n=25) will be injected with placebo. The primary aim is to evaluate the safety of the drug in these young non-diabetic children. The secondary aim is to evaluate beta-cell capacity in participating children before and treatment with alum-formulated GAD65 injections to find out if this treatment may prevent beta-cell destruction before the clinical onset of type 1 diabetes. The study is still recruiting and blinded, but no serious adverse events have been reported in 37 children recruited so far. Two children have developed diabetes. This study is the first attempt to use alum-formulated GAD65 to prevent disease onset in high-risk individuals.

Antigen-specific immunotherapy – intervention trials

Diamyd Therapeutics AB is currently carrying out a phase III clinical trial with 230 patients in Europe (NCT00723411) and an equal number in the US (DIAPREVENT - NCT00751842) (<http://www.diamyd.com/>). The inclusion criteria stipulate disease duration of only three months. The phase III studies are three-armed with one arm with two doses of alum-formulated GAD65 (day 1 and 30), one arm with four doses of alum-formulated GAD65 (day 1, 30, 90 and 270) and one arm with placebo. The study will be evaluated after 15 months of follow up and results are expected during 2011.

DiaPep277

DiaPep277 is a peptide with immunomodulatory properties. The mechanisms of action are unknown but are thought to stop or reduce the autoimmune attack on the pancreatic islet beta cells. The humanized DiaPep277 by Andromeda Biotech Ltd. was found to provide preservation of beta-cell function and enhance the levels of C-peptide in a group of trials [61]. Administration of DiaPep277 seemed safe and may have had beneficial effects on C-peptide levels in some patients with type 1 diabetes. Current trials (NCT01103284 and NCT00644501) will examine the effect of DiaPep277 on preservation of beta-cell function, as defined by meal-stimulated c-peptide. Adults (>20 years) with newly diagnosed (<6 months) type 1 diabetes will be treated with 10 injections of DiaPep277 or placebo over a two-year treatment and follow-up period.

Combination Therapies

The search for a single drug that can modulate the prodrome of islet autoimmunity in type 1 diabetes seems to continue. However, it is often questioned to what extent a single agent may be able to modify or retard the progression to clinical onset. Number of autoantibodies is thought to be the best predictor of clinical onset [1, 2]. As the immune system is like not to be able to count the number of islet autoantibodies, this arbitrary phenomenon is more likely to reflect that only one islet autoantibody indicate that an autoreactive signaling pathway has been established against only one autoantigen. The appearance of a second autoantibody would indicate that yet another autoreactive signaling pathway has been established against another autoantigen. Each autoreactive-signaling pathway may have different contribution to the disease pathogenesis and progression to clinical onset. For example, insulin autoantibodies are less predictive of type 1 diabetes compared to GAD65 autoantibodies [2]. Therefore, combination therapy is proposed for antigen-specific immunotherapy [62]. Current proposals include the combination of two immunomodulatory agents, non-specific antibody-based immunotherapy such as anti-CD3 monoclonal antibodies and antigen-specific immunotherapy such as proinsulin peptide or GAD65. This combination may permit a reduction in the dose or the duration of one or both of the used drugs and maximize benefits or minimize adverse effects. The alternative would be to use

current experience with alum-formulated GAD65 and test a combination therapy with alum-formulated human proinsulin (alternatively IA-2 or ZnT8) in a factorial trial design (Table 1). This design would allow the advantage of recruiting subjects who have significant autoantibodies against both GAD65 and proinsulin to evaluate effects of the individual autoantigen, the autoantigens combined, all in relation to the placebo treated subjects.

Future directions

Autoantigen-specific immunotherapy or so-called “diabetes vaccination” of individuals with established islet autoimmunity or at the time of clinical diagnosis of type diabetes will depend on the success of ongoing prevention and intervention trials. Since 1976, many newly diagnosed type 1 diabetes patients have been given immunosuppressive agents. None of the single drug approaches have preserved beta-cell function long term. Transient effects have been reported but the benefits have been limited. Many patients have been exposed to immunosuppression, which may have future adverse effects. Insulin replacement therapy has been continued. Antigen-specific immunotherapy (ASI) with alum-formulated GAD65 alone showed a reduced loss of C-peptide after diagnosis of diabetes. Future direction may include a combination therapy with immunosuppression combined with ASI to test whether tolerance induction would be more efficient. Alternatively, ASI with multiple autoantigens may increase efficacy. Provided the treatment is safe, it cannot be excluded that the simultaneous administration of GAD65, IA-2, ZnT8 and proinsulin may be more efficacious than GAD65 alone. The route of administration needs further exploration. Oral insulin was tested but what about oral GAD65? Should alum-GAD65 tested together with alum-formulated proinsulin? Safe trials may provide a novel approach to dissect the mechanisms by which the human immune system responds to ASI with autoantigens.

Acknowledgments

The authors are supported by the Swedish Child Diabetes Foundation, Swedish Research Council, Swedish Diabetes Association, Knut & Alice Wallenberg Foundation, UMAS Funds, the Skåne County Council of Research and Development and the National Institutes of Health (DK063861).

References

1. DPT-1. Effects of insulin in relatives of patients with type 1 diabetes mellitus. The New England Journal of Medicine. 2002; 346:1685–1691. [PubMed: 12037147]
2. Orban T, Sosenko JM, Cuthbertson D, et al. Pancreatic islet autoantibodies as predictors of type 1 diabetes in the Diabetes Prevention Trial-Type 1. Diabetes Care. 2009; 32:2269–2274. [PubMed: 19741189]
3. Pipeleers D, Ling Z. Pancreatic beta cells in insulin-dependent diabetes. Diabetes Metab Rev. 1992; 8:209–227. [PubMed: 1292912]
4. Wallensteen M, Dahlquist G, Persson B, Landin-Olsson M, Lernmark A, Sundkvist G, Thalme B. Factors influencing the magnitude, duration, and rate of fall of B-cell function in type 1 (insulin-dependent) diabetic children followed for two years from their clinical diagnosis. Diabetologia. 1988; 31:664–669. [PubMed: 3069534]
5. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, et al. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. N Engl J Med. 2009; 361:2143–2152. [PubMed: 19940299]
6. Ludvigsson J, Faresjo M, Hjorth M, et al. GAD treatment and insulin secretion in recent-onset type 1 diabetes. N Engl J Med. 2008; 359:1909–1920. [PubMed: 18843118]
7. Porksen S, Laborie LB, Nielsen L, et al. Disease progression and search for monogenic diabetes among children with new onset type 1 diabetes negative for ICA, GAD- and IA-2 Antibodies. BMC Endocr Disord. 2010; 10:16. [PubMed: 20863361]
8. Naik RG, Brooks-Worrell BM, Palmer JP. Latent autoimmune diabetes in adults. J Clin Endocrinol Metab. 2009; 94:4635–4644. [PubMed: 19837918]

9. Leslie RD, Kolb H, Schloot NC, et al. Diabetes classification: grey zones, sound and smoke: Action LADA 1. *Diabetes Metab Res Rev.* 2008; 24:511–519. [PubMed: 18615859]
10. Jensen RA, Gilliam LK, Torn C, et al. Multiple factors affect the loss of measurable C-peptide over 6 years in newly diagnosed 15- to 35-year-old diabetic subjects. *J Diabetes Complications.* 2007; 21:205–213. [PubMed: 17616349]
11. Hovorka R. Closed-loop insulin delivery: from bench to clinical practice. *Nat Rev Endocrinol.* 2010
12. Pipeleers D, Chintinne M, Denys B, Martens G, Keymeulen B, Gorus F. Restoring a functional beta-cell mass in diabetes. *Diabetes Obes Metab.* 2008; 10 Suppl 4:54–62. [PubMed: 18834433]
13. Lakey JR, Mirbolooki M, Shapiro AM. Current status of clinical islet cell transplantation. *Methods Mol Biol.* 2006; 333:47–104. [PubMed: 16790847]
14. Vendrame F, Pileggi A, Laughlin E, et al. Recurrence of type 1 diabetes after simultaneous pancreas-kidney transplantation, despite immunosuppression, is associated with autoantibodies and pathogenic autoreactive CD4 T-cells. *Diabetes.* 2010; 59:947–957. [PubMed: 20086230]
15. Uibo R, Lernmark A. GAD65 autoimmunity-clinical studies. *Adv Immunol.* 2008; 100:39–78. [PubMed: 19111163]
16. Geffner ME, Lippe BM. The role of immunotherapy in type I diabetes mellitus. *West J Med.* 1987; 146:337–343. [PubMed: 3554759]
17. Skyler JS. Immune intervention studies in insulin-dependent diabetes mellitus. *Diabetes Metab Rev.* 1987; 3:1017–1035. [PubMed: 3315520]
18. Skyler JS. Immunomodulation for type 1 diabetes mellitus. *Int J Clin Pract Suppl.* 2010:59–63. [PubMed: 20377666]
19. Bougneres PF, Carel JC, Castano L, et al. Factors associated with early remission of type I diabetes in children treated with cyclosporine. *N Engl J Med.* 1988; 318:663–670. [PubMed: 3125434]
20. 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]
21. Martin S, Scherthaner G, Nerup J, et al. Follow-up of cyclosporin A treatment in type 1 (insulin-dependent) diabetes mellitus: lack of long-term effects. *Diabetologia.* 1991; 34:429–434. [PubMed: 1884902]
22. Kaufman A, Herold KC. Anti-CD3 mAbs for treatment of type 1 diabetes. *Diabetes Metab Res Rev.* 2009; 25:302–306. [PubMed: 19319985]
23. Jaume JC, Parry SL, Madec AM, Sonderstrup G, Baekkeskov S. Suppressive effect of glutamic acid decarboxylase 65-specific autoimmune B lymphocytes on processing of T cell determinants located within the antibody epitope. *J Immunol.* 2002; 169:665–672. [PubMed: 12097368]
24. Steed J, Gilliam LK, Harris RA, Lernmark A, Hampe CS. Antigen presentation of detergent-free glutamate decarboxylase (GAD65) is affected by human serum albumin as carrier protein. *J Immunol Methods.* 2008; 334:114–121. [PubMed: 18353353]
25. Buonaguro FM, Tornesello ML, Buonaguro L. Virus-like particle vaccines and adjuvants: the HPV paradigm. *Expert Rev Vaccines.* 2009; 8:1379–1398. [PubMed: 19803760]
26. La Torre D, Lernmark A. Immunology of beta-cell destruction. *Adv Exp Med Biol.* 2010; 654:537–583. [PubMed: 20217514]
27. Wenzlau JM, Frisch LM, Gardner TJ, Sarkar S, Hutton JC, Davidson HW. Novel antigens in type 1 diabetes: the importance of ZnT8. *Curr Diab Rep.* 2009; 9:105–112. [PubMed: 19323954]
28. Hampe CS, Kockum I, Landin-Olsson M, et al. GAD65 antibody epitope patterns of type 1.5 diabetic patients are consistent with slow-onset autoimmune diabetes. *Diabetes Care.* 2002; 25:1481–1482. [PubMed: 12145258]
29. Bekris LM, Jensen RA, Lagerquist E, et al. GAD65 autoantibody epitopes in adult patients with latent autoimmune diabetes following GAD65 vaccination. *Diabet Med.* 2007; 24:521–526. [PubMed: 17367313]
30. Patterson CC, Dahlquist GG, Gyurus E, Green A, Soltesz G. Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: a multicentre prospective registration study. *Lancet.* 2009; 373:2027–2033. [PubMed: 19481249]

31. Concannon P, Rich SS, Nepom GT. Genetics of type 1A diabetes. *N Engl J Med.* 2009; 360:1646–1654. [PubMed: 19369670]
32. Roivainen M, Klingel K. Virus infections and type 1 diabetes risk. *Curr Diab Rep.* 2010; 10:350–356. [PubMed: 20680525]
33. Schlosser M, Mueller PW, Torn C, Bonifacio E, Bingley PJ. Diabetes Antibody Standardization Program: evaluation of assays for insulin autoantibodies. *Diabetologia.* 2010; 53:2611–2620. [PubMed: 20871974]
34. Bonifacio E, Yu L, Williams AK, et al. Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for national institute of diabetes and digestive and kidney diseases consortia. *J Clin Endocrinol Metab.* 2010; 95:3360–3367. [PubMed: 20444913]
35. Bonifacio E, Mayr A, Knopff A, Ziegler AG. Endocrine autoimmunity in families with type 1 diabetes: frequent appearance of thyroid autoimmunity during late childhood and adolescence. *Diabetologia.* 2009; 52:185–192. [PubMed: 19018508]
36. Stene LC, Oikarinen S, Hyoty H, et al. Enterovirus infection and progression from islet autoimmunity to type 1 diabetes: the Diabetes and Autoimmunity Study in the Young (DAISY). *Diabetes.* 2011; 59:3174–3180. [PubMed: 20858685]
37. Nanto-Salonen K, Kupila A, Simell S, et al. Nasal insulin to prevent type 1 diabetes in children with HLA genotypes and autoantibodies conferring increased risk of disease: a double-blind, randomised controlled trial. *Lancet.* 2008; 372:1746–1755. [PubMed: 18814906]
38. Larsson HE, Hansson G, Carlsson A, et al. Children developing type 1 diabetes before 6 years of age have increased linear growth independent of HLA genotypes. *Diabetologia.* 2008; 51:1623–1630. [PubMed: 18592208]
39. The Environmental Determinants of Diabetes in the Young (TEDDY) study: study design. *Pediatr Diabetes.* 2007; 8:286–298. [PubMed: 17850472]
40. In't Veld P, Lievens D, De Grijse J, et al. Screening for insulinitis in adult autoantibody-positive organ donors. *Diabetes.* 2007; 56:2400–2404. [PubMed: 17563060]
41. Mackay IR, Rowley MJ. Autoimmune epitopes: autoepitopes. *Autoimmun Rev.* 2004; 3:487–492. [PubMed: 15546795]
42. Mallone R, van Endert P. T cells in the pathogenesis of type 1 diabetes. *Curr Diab Rep.* 2008; 8:101–106. [PubMed: 18445351]
43. Csorba TR, Lyon AW, Hollenberg MD. Autoimmunity and the pathogenesis of type 1 diabetes. *Crit Rev Clin Lab Sci.* 2010; 47:51–71. [PubMed: 20545565]
44. Karlsten AE, Hagopian WA, Grubin CE, et al. Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10. *Proc Natl Acad Sci U S A.* 1991; 88:8337–8341. [PubMed: 1924293]
45. 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]
46. Kaufman DL, Clare-Salzler M, Tian J, et al. Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature.* 1993; 366:69–72. [PubMed: 7694152]
47. Tian J, Clare-Salzler M, Herschenfeld A, et al. Modulating autoimmune responses to GAD inhibits disease progression and prolongs islet graft survival in diabetes-prone mice. *Nat Med.* 1996; 2:1348–1353. [PubMed: 8946834]
48. Tisch R, Liblau RS, Yang XD, Liblau P, McDevitt HO. Induction of GAD65-specific regulatory T-cells inhibits ongoing autoimmune diabetes in nonobese diabetic mice. *Diabetes.* 1998; 47:894–899. [PubMed: 9604865]
49. Plesner A, Worsaae A, Dyrberg T, Gotfredsen C, Michelsen BK, Petersen JS. Immunization of diabetes-prone or non-diabetes-prone mice with GAD65 does not induce diabetes or islet cell pathology. *J Autoimmun.* 1998; 11:335–341. [PubMed: 9776711]
50. Lernmark A, Agardh CD. Immunomodulation with human recombinant autoantigens. *Trends Immunol.* 2005; 26:608–612. [PubMed: 16153889]

51. Agardh CD, Cilio CM, Lethagen A, et al. Clinical evidence for the safety of GAD65 immunomodulation in adult-onset autoimmune diabetes. *J Diabetes Complications*. 2005; 19:238–246. [PubMed: 15993359]
52. Agardh CD, Lynch KF, Palmer M, Link K, Lernmark A. GAD65 vaccination: 5 years of follow-up in a randomised dose-escalating study in adult-onset autoimmune diabetes. *Diabetologia*. 2009; 52:1363–1368. [PubMed: 19404608]
53. Oak S, Gilliam LK, Landin-Olsson M, et al. The lack of anti-idiotypic antibodies, not the presence of the corresponding autoantibodies to glutamate decarboxylase, defines type 1 diabetes. *Proc Natl Acad Sci U S A*. 2008; 105:5471–5476. [PubMed: 18367670]
54. Orqvist E, Brooks-Worrell B, Lynch K, et al. Changes in GAD65Ab-specific antiidiotypic antibody levels correlate with changes in C-peptide levels and progression to islet cell autoimmunity. *J Clin Endocrinol Metab*. 2010; 95:E310–E318. [PubMed: 20685858]
55. Ludvigsson J. The role of immunomodulation therapy in autoimmune diabetes. *J Diabetes Sci Technol*. 2009; 3:320–330. [PubMed: 20144363]
56. Ludvigsson J, Hjorth M, Cheramy M, et al. 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]
57. Cheramy M, Skoglund C, Johansson I, Ludvigsson J, Hampe CS, Casas R. GAD-alum treatment in patients with type 1 diabetes and the subsequent effect on GADA IgG subclass distribution, GAD65 enzyme activity and humoral response. *Clin Immunol*. 2010; 137:31–40. [PubMed: 20580618]
58. Hjorth M, Axelsson S, Ryden A, Faresjo M, Ludvigsson J, Casas R. GAD-alum treatment induces GAD65-specific CD4+CD25highFOXP3+ cells in type 1 diabetic patients. *Clin Immunol*. 2010; 138:117–126. [PubMed: 21044870]
59. Fourlanos S, Perry C, Gellert SA, et al. Evidence That Nasal Insulin Induces Immune Tolerance to Insulin in Adults With Autoimmune Diabetes. *Diabetes*. 2010
60. Skyler JS, Krischer JP, Wolfsdorf J, et al. Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial--Type 1. *Diabetes Care*. 2005; 28:1068–1076. [PubMed: 15855569]
61. Schloot NC, Meierhoff G, Lengyel C, et al. 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]
62. Boettler T, Herrath MV. Type 1 diabetes vaccine development: Animal models vs. humans. *Hum Vaccin*. 2011; 7

Figure 1. The possible role of Helper T cells, B lymphocytes and plasma cells in autoantigen presentation and formation of autoantibodies

B cells express B-cell receptors (BCR). The BCR are membrane associated antibodies, which may bind and internalize an autoantigen such as (pro)insulin or GAD65. The autoantigen is processed and presented (green bar) on HLA Class II molecules (yellow box) on the surface of the B cell. Helper T cells expressing T cell receptor specific (red and blue symbols of the alpha and beta chains) for the trimolecular complex of HLA and green autoantigen peptide is activated. The activated Helper T cell will produce lymphokines that in turn will activate the B cell to begin producing autoantibodies. Mitosis and differentiation will lead to plasma cells that continue to produce the autoantibodies.

Table 1

Islet autoantigens in type 1 diabetes.

Autoantigen	Protein	Function	ASI in humans
(Pre)(pro)insulin	Precursor and processed product	Hormone	Oral insulin may preserve beta-cell function
GAD65	Gutamic acid decarboxylase - Mr 65,000 isoform	GABA producing enzyme	Alum-formulated GAD65 preserves beta-cell function
IA-2	Islet antigen-2	tyrosine phosphatase-like protein - unknown function	To be tested
ZnT8	Zn T8 transporter	One of several Zn transporters but specific to the beta cells	To be tested

ASI is Antigen-Specific Immunotherapy

Table 2

Factorial design of a proposed trial to test combination antigen-specific immunotherapy.

Group	Autoantigen A	Autoantigen B
I	placebo	placebo
II	GAD65	placebo
III	Proinsulin	placebo
IV	GAD65	Proinsulin

Candidate autoantigens include GAD65, proinsulin, IA-2 and ZnT8 transporter.