Prevention of Type 1A Diabetes: Update

Pnina Vardi MD[1,2], Ilana Koren MD[2] and Konstantin Bloch PhD[1]

[1] Laboratory for the Research of Diabetes and Obesity, Felsenstein Medical Research Center (Beilinson Campus), Petah Tiqva, Israel
Affiliated to Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel
[2] Childhood Diabetes Unit, Lin Medical Center, Clalit Health Services, Haifa and Western Galilee District, Israel

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Since the affirmation that type 1A diabetes is an autoimmune disease, substantial efforts have been invested to predict and prevent the onset of the disease, using various immune-related markers and treatments. Detection of autoantibodies to the pancreatic islets in non-diabetic subjects is still considered the most reliable marker of active islet autoimmunity and is therefore used to recruit subjects at high risk for developing T1DM to preventive trials. Unfortunately, no long-term success was obtained in any of these trials, suggesting that either the specific drugs or the dose administered were not adequate. Failure to prevent the disease can also be the result of inappropriate timing of the intervention strategy – when islet autoimmunity reached an irreversible stage or due to an incorrectly tailored treatment modality. Some of these possibilities are currently under consideration, and various immune-mediated drugs are being prepared for new T1DM prevention trials.

Natural history of type 1A diabetes

Type 1A (immune-mediated) diabetes mellitus in humans and animals has been the target of large intensive studies. Unlike animal models of T1DM where the disease could be prevented with a number of compounds, no success has been achieved in humans [Table 1]. It is believed that T1DM is a chronic, progressive autoimmune disease that develops in genetically prone individuals and animals [1]. After the activation of islet autoimmunity, beta-cell destruction is followed by mass reduction and dysfunction of islets, as reflected by progressive, higher individual fasting blood levels of glucose, hemoglobin A1c (unpublished data), loss of first-phase insulin secretion, and impaired tolerance to oral glucose load [1,2].

Predictive markers

Islet autoantibodies

In humans, the best marker of islet autoimmunity is the presence of autoantibodies reacting with multiple islet autoantigens and epitopes, and the most useful autoantibody assays are those designed to detect three major islet antigens, namely insulin, glutamic acid decarboxylase, and ICA512 (IA-2). The majority of subjects expressing only a single autoantibody do not progress to diabetes, while 60% of those expressing two or more autoantibodies are at risk of developing diabetes over the next decade [3–5].

Genetics of T1DM

It is believed that T1DM is a polygenic disease in which the contribution of the human leukocyte antigen region is by far the highest, exceeding 50%. Recent genome-wide searches for T1DM susceptibility genes have provided preliminary evidence for the existence of at least 18 loci associated with T1DM [6]. One of the various approaches for understanding the genetics of T1DM is to evaluate the empiric power and efficiency of mapping the disease in large multiplex T1DM families. We performed such a study in a

Table 1. Trials in newly diagnosed T1DM and autoantibody-positive individuals

Intervention	Status	Outcome	Countries
CsA	Ended	1 year success	Canada, France
BCG	Ended	Failure	USA, Israel*
Anti-CD3	Ended	1 year success	USA, Belgium
Nasal insulin	Ongoing	Unknown	Finland
Two immuno-suppressants	Preparing to recruit	Unknown	USA
Avoidance of cow's milk	Ongoing	Unknown	Finland
Parenteral insulin DPT-1	Ended	Failure	USA**
Oral insulin DPT-1	Ended	Failure	USA**
Nicotinamide	Ended	Failure	Europe**
P277	Ongoing	Initial success	Israel
GAD peptides	Preparing to recruit	Unknown	USA

* Unpublished results
** Multicenter study

T1DM = type 1A diabetes mellitus

Bedouin family with multiplex cases of T1DM (20 affected individuals) and found a linkage with a locus on chromosome 10, named *IDDM17* [7]. The usefulness of a homogeneous population was also evaluated in patients of Yemenite origin, who unlike other communities in Israel were found to have a certain insulin-dependent diabetes susceptibility genotype with an *exceptionally* high T1DM-predisposing effect [8]. Although the genetic background was never considered in T1DM prevention trials, the protective DQB1*0602 allele was used as an excluding criterion. In some populations in which a high odds score of genetics exceeds the predictive value of autoantibodies, genetics can be used for recruitment of subjects to early intervention trials designed to intervene prior to evidence of active autoimmunity.

Prevention trials

In general, the preventive strategies aim to confer protection from overt diabetes in subjects at high risk of developing T1DM, to preserve residual beta-cell mass in recent-onset T1DM patients, to prevent or delay insulin dependency in patients with latent autoimmune diabetes in the adult (LADA) and to use successful drugs to prevent recurrence of autoimmunity in patients transplanted with pancreatic islet cells. Various preventive approaches have been taken in populations at high risk of developing T1DM, but unfortunately, the three major, multicenter prevention trials: parenteral insulin, oral insulin (DPT-1) and the oral nicotinamide trial (ENDIT), failed to prevent progression of the disease.

Our experience with parenteral insulin and Bacillus Calmette-Guerin showed that positive and prolonged protective effects were obtained in individuals who kept a restricted diet and practiced intensive physical activity. This observation was valid for both recent-onset diabetic patients and for antibody-positive individuals, suggesting a substantial effect of insulin sensitivity on islet protection and survival.

Initial intervention trials in T1DM

Following the discovery of the autoimmune background in T1DM, several immune-based trials to intervene in the destructive process of the islets have been designed, including steroids [9], cyclosporine A, and azathioprine [10]. In all of them, various degrees of beta-cell protection were observed but unfortunately not maintained over a long follow-up. In addition, the potential and actual side effects of these drugs – nephrotoxicity with CsA, hepatotoxicity and bone marrow suppression with azathioprine – could not justify their continued use. Despite their failure, it was learned that the natural history of T1DM could be modified.

- **Parenteral insulin:** Parenteral insulin has been shown to protect against T1DM in animal models of the disease [11]. The protective effect is believed to result either from injection-induced, re-regulation of the immune system response, and/or from lower level of autoimmune stimulation by insulin antigen due to lower insulin production (beta-cell rest). Initial preliminary studies performed in autoantibody-positive, first-degree relatives of T1DM patients showed that parenteral insulin administration is T1DM protective [12]. In T1DM animals, the protective effect of insulin was shown to result from induction of immune regulation with a shift versus a Th2/interleukin 4-mediated tolerant response, and beta-cell repair and regeneration. The large multicenter insulin prevention trial, named Diabetes Prevention Trial-Type 1, or DPT-1, screened 104,000 relatives of T1DM subjects, and enrolled subjects with at least a 26%, 5 year risk of developing T1DM. About half of those with a moderate risk of 26–50% were prescribed oral insulin, and half received a placebo. However, failure of the parenteral arm to protect against T1DM was announced by the DPT-1 in 2002 [13]. Administration of metabolically inactive insulin such as the beta-chain of the insulin molecule also confers T1DM protection in mice [14]. Splenocytes from non-obese diabetic mice immunized with beta-chain insulin only were found to activate regulatory T cells and reduce interferon-gamma level in pancreatic islets. T1DM was also delayed with somatostatin treatment, an agent that suppresses endogenous insulin production, suggesting that both immunologic and metabolic factors contribute to the protective effect of insulin in NOD mice.
- **Oral insulin:** Oral administration of relevant autoantigens has been reported to delay or suppress the onset of autoimmune T1DM. While oral insulin was found to reduce the severity of lymphocytic infiltration in the pancreatic islets of the NOD mouse, a very large amount of insulin is needed in order to be effective. A new strategy using small amounts of insulin conjugated to a mucosal carrier protein such as insulin-MCP (cholera toxin β-subunit) is currently being developed [15].

  Despite the promising expectations, a failure of (DPT-1) oral insulin therapy to prevent overt T1DM was declared at the annual meeting of the American Diabetes Association in New Orleans, 2003.

Other drugs influencing the immune system

In addition to CsA, other promising immunomodulators, such as BCG, were studied for their potential to intervene with the autoimmune process of islet destruction, with negative results [16].

CD40-CD40L interactions are responsible for the production of antibodies to T cell-dependent antigens, formation of germinal centers, and immunoglobulin isotype switching. The effect of CD40-CD40L blockade has been shown to promote the acceptance of islet allografts in mice and non-human primates [17]. Anti-CD-80 antibody was shown to be effective in blocking T cell activation, IL-2 secretion with no severe adverse effects, and no change in CD4, CD8 or CD20. Preclinical studies showed that monoclonal antibody against CD3 could reverse hyperglycemia and induce tolerance to recurrent diabetes. Recently, administration of a single 14 day course of humanized monoclonal antibody against CD3-hOKT3γ1 (Ala-Ala) was shown to maintain or improve insulin production after 1 year in patients with recent-onset T1DM [18].

CsA = cyclosporine A

NOD = non-obese diabetic
BCG = Bacillus Calmette-Guerin
IL = interleukin

Antigen-based therapy

Insulin B9-23 peptide

This is a dominant epitope of the insulin autoantigen, recognized by the immune system of the NOD mice. A series of peptide ligands designed to block or modify its immune effect has been proposed, including a novel peptide, NBI-6024, that produces a strong protective Th2 type effect. Unfortunately, administration of B9-23, in the absence of adjuvant, induces a dramatic humoral response leading to fatal anaphylaxis in NOD mice [19].

GAD65

In the NOD mouse, GAD65-reactive T cells have been shown to be critically involved in the early destruction of the pancreatic islet. Injections of GAD65 and GAD peptides were shown to induce tolerance and prevention of diabetes, while immunization with GAD65 did not cause islet cell pathology [20]. The induction of both the passive and the active immune response has been studied and found to successfully protect against T1DM in the NOD mouse. Accordingly, intervention approaches using subcutaneous injections of GAD peptides in diabetic patients with LADA are currently being investigated.

Peptide 277

Heat shock protein 60 constitutes a major activating signal of the immune system, playing a major role in the pathogenesis of various autoimmune disorders, and was found to be associated with T1DM in both diabetic patients and animals. A peptide-based compound vaccine for T1DM that was developed suppressed the Th1 responses in animal models. An initial double-blind study that used the vaccine in T1DM patients over the age of 16 with recent onset of T1DM showed a protective effect on residual alpha cells [21]. However, no data on the effect of this vaccine in younger patients, in whom the destructive process is more aggressive, are yet available.

Nicotinamide and dietary manipulation

Nicotaninamide is a component of NAD- and NADP+ and is an essential dietary constituent, which was shown to protect beta cells against a variety of toxic stimuli, including that of alloxan, streptozocin, IL-1, interferon-alpha, and tumor necrosis factor-alpha. It reduced the severity of insulitis in young NOD mice and stimulated beta-cell regeneration in pancreatectomized rats [22]. Unfortunately, like other promising islet-protective drugs, its expected T1DM beneficial effects failed in humans [23]. By not introducing cow's milk to the diet of neonates at high genetic risk reduced the appearance of autoantibody, but a longer follow-up will disclose whether it also prevents T1DM development.

Conclusion

Despite the enthusiasm that has swept the world in anticipation of a cure for type 1 diabetes, there is no indication that this expectation has been fulfilled. Still, much has been learned and achieved. It is likely that, in the future, intervention strategies will use different drug combinations, according to the diagnosed stage of T1DM events, expected aggressiveness of the disease, population ethnicity and genetics, timing (uterine, post-uterine), antibody presence and number, etc. In addition, new, more specific, genetically based drugs, combined with an optimal dose of non-immune islet protectors, will hopefully complete the measures intended to block the disease and allow successful islet transplantation in chronic patients with no residual beta cells.

GAD = glutamic acid decarboxylase

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Correspondence: Dr. P. Vardi, Laboratory for the Research of Diabetes and Obesity, Felsenstein Medical Research Center, Petah Tiqva 49101, Israel.
email: pvardi@post.tau.ac.il