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59 Future Drug Treatment for Type 1 Diabetes

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Keypoints

- Insulin replacement therapy is considered the only effective and feasible treatment for type 1 diabetes mellitus (T1DM) as only insulin is capable of reversing the metabolic disturbances and restoring a near-normal quality of life in patients with T1DM.
- Despite rigorous measures and major advances in health care provided for patients with T1DM, increased morbidity and mortality are still common from complications, which commonly develop within 10–12 years after clinical onset.
- Advances in the understanding of the natural history of T1DM and increased abilities to predict the disease have made it possible to design and implement prevention and intervention clinical trials.
- Clinical trials are aimed at: (a) preventing the initiation of islet autoimmunity (primary prevention); (b) reducing autoimmune β-cell killing and progression to clinical diabetes (secondary prevention); or (c) suppressing or modulating the immune response in order to halt β-cell killing and enhance β-cell regeneration (tertiary prevention or intervention).
- Several trials were implemented or are currently ongoing with dietary manipulation, parenteral or oral insulin or immune-suppressing or immune-modulating agents with the aim of preventing the disease or retarding its progression.
- The search for safe, effective and feasible drugs to prevent or cure T1DM is still ongoing. So far, immune modulation with alum-formulated GAD65 has been shown to be the most promising intervention to reduce the loss of β-cells. Anti-CD3 monocloncal autoantibodies also showed some benefits in patients with newly diagnosed T1DM.

Introduction

The pathophysiologic mechanisms in type 1 diabetes mellitus (T1DM) are proposed to progress over several stages, in which autoimmunity is triggered in genetically predisposed individuals and in which β-cell killing by cellular immunity is activated, leading to insulin deficiency [1]. The autoimmune insult, which leads to selective killing of β-cells, may take months to years to develop. Throughout this autoimmune prodrome several genetic, autoimmune and biochemical markers may predict the disease prior to clinical onset. Once the clinical disease is established, the patient will be dependent on exogenous insulin and will require strict control to sustain euglycemia and minimize complications. Shortly after clinical onset of diabetes and initiation of insulin treatment, the remaining β-cells often increase their capacity to produce insulin, thereby decreasing the need for insulin treatment. This period of low insulin requirement, known as "partial remission" or the "honeymoon" period, may last for months or even years and is advantageous for the patient because it simplifies treatment and improves metabolic control, leading to less risk of long-term complications [2]. The "honeymoon" period seems to be longer and appears more frequently in older children and adults than in children diagnosed at a younger age [3].

Several trials seeking to prevent or delay the clinical onset of T1DM in genetically predisposed individuals as well as attempting to spare or even improve residual β-cell function in recently diagnosed patients are planned, implemented or currently ongoing.

Short history of type 1 diabetes prevention trials

Several approaches to modulate the immune system have been tested to prolong the partial remission in newly diagnosed patients. In the 1980s, plasmapheresis was performed in patients newly diagnosed with T1DM. A tendency of improved residual β-cell function and metabolic control was observed [4]. Later, several attempts with immune-modulating drugs were carried out. In order to suppress the immune response against the β-cells, small doses of cyclosporine were administered in a pilot study to autoantibody-positive first-degree relatives (FDR) of patients with T1DM with decreased first-phase insulin release (FPIR). The drug increased the FPIR, suggesting that cyclosporine may delay the onset of T1DM in glucose-intolerant siblings [5]. Cyclosporine was further tested in the Canadian–European Randomized Control Trial [6] as well as in series of controlled or uncontrolled smaller studies [5,7]. It was found that cyclosporine temporarily

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reduced insulin requirement and enhanced endogenous β-cell function in patients with newly diagnosed T1DM [6]. Although cyclosporine had some effect on preserving β-cell function, further clinical trials were abandoned because of severe kidney lesions [6,8]. Other immunosuppressive drugs such as azathioprine were also tested, either alone [9] or with prednisolone [10]. These immunosuppression trials had verified the immunopathogenic basis of T1DM, indicated by improved indicators of β-cell function in some trials; however, the outcomes were hard to maintain because of concerns about serious side effects [5,7].

Concerns about safety and sustainability of therapeutic effects directed attention towards the search for other preventive agents, with the focus on initiating preventive trials based on the stages of disease progression; however, our understanding of the natural history of T1DM made it possible to identify highly predictive markers (genetic, immunologic and metabolic) and recognize putative etiologic factors and possible intervention methods.

Several studies tested non-antigen-specific agents as possible preventive agents. The Bacille Calmette–Guérin (BCG) vaccine was found to prevent T1DM in experimental animals such as the non-obese diabetic (NOD) mice [11] and the bio-breeding (BB) rat [12]; however, similar results were not achieved in a group of human pilot studies [13,14]. The histamine antagonist, ketotifen [15], used orally in a pilot study in islet cell antibody (ICA) positive children with low FPIR showed no significant effect. Nicotinamide (vitamin B_3) protected β-cells in rodent studies [16] and so the effect of nicotinamide was tested in the European Nicotinamide Diabetes Intervention Trial (ENDIT) [17]. Nicotinamide was administered orally to ICA-positive FDR of patients with T1DM but the drug did not prevent progression to diabetes. High doses of nicotinamide were tested in other efficacy studies such as the DENIS [18] but this study also failed to show beneficial effects among high-risk children. Additionally, a group of pilot studies tested the combination of nicotinamide with cyclosporine [19], vitamin E [20] or intensive insulin regimen [21], none of which showed a preventive effect. These studies showed that nicotinamide could not protect against the loss of β-cells in high-risk subjects and overall no significant beneficial effects were detected.

Insulin, as an antigen-specific agent, has been tested through different routes, parenteral [22], oral [23] or intranasal [24,25] in genetically predisposed individuals, FDR and patients with T1DM in several trials. These trials showed no effect on disease progression or the rate of β-cell loss; however, subanalysis from the oral arm of the Diabetes Prevention Trial-1 (DPT-1) revealed a statistically significant delay in onset of T1DM in subjects with high titer insulin autoantibodies (IAA). A repeat study of the oral insulin trial has therefore been initiated.

Several prevention and intervention trials with long follow-up durations, some even including a combination of therapies, are now ongoing or planned. These trials use different approaches, with goals of preventing the triggering of autoimmunity (primary prevention), halting β-cell destruction (secondary prevention) or preserving remaining β-cells and enhancing their regeneration (tertiary prevention). This chapter presents an evidence-based review of the main ongoing drug clinical trials in subjects at-risk for or in those with newly diagnosed T1DM.

Prediction

Since the late 1970s, several studies have indicated that it is possible to predict T1DM among FDR through testing for human leukocyte antigen (HLA) class II genes and IAA. Several such studies utilized HLA genotyping, islet autoantibody analyses and biochemical indicators, as predictive markers are currently ongoing (Table 59.1). These prospective screening studies, may

Table 59.1 Some of the recent or ongoing screening studies for T1DM in first-degree relatives or high-risk children in the general population.

Study	Screening period	Screening population	Country
German BABYDIAB	1989–2000	Offspring of patients with diabetes	Germany
Australian BABYDIAB	1993	First-degree relatives	Australia
Diabetes Autoimmunity Study in the Young (DAISY)	1993–	First-degree relatives and high-risk children in general population	USA (Colorado)
Diabetes Prediction and Prevention Program (DIPP)	1994–	High-risk children in general population	Finland
Diabetes Evaluation in Washington (DEW-IT)	1995–	High-risk children in general population	USA (Washington)
Prospective Assessment of Newborns for Diabetes Autoimmunity Study (PANDA)	1997–	High-risk children in general population	USA (Florida)
All Babies in south-east Sweden (ABIS)	1997–1999	General population	Sweden
Diabetes Prediction in Skåne (DiPiS)	2000–2004	High-risk children in general population	Sweden
Environmental Triggers of Type 1 Diabetes Study (MIDIA)	2001–	High-risk children in general population	Norway
The Environmental Determinants of Diabetes in the Young (TEDDY)	2004–	Multicenter study of high-risk children in general population	Sweden (Skåne), Finland, Germany, USA (Colorado, Florida, Georgia, Washington)

allow us to define the environmental factors that may trigger islet autoimmunity or accelerate the development of T1DM in subjects with islet autoimmunity. These studies should make it possible to follow the disease process from initiation to the clinical onset of T1DM to help us understand the course of events during β-cell autoimmunity. At the same time, our knowledge about patterns of disease markers during subclinical disease and the predictive value of islet cell autoantibodies should be improved.

Predicting a disease at an early stage of pathologic insult is a prerequisite for attempts to prevent disease progression and initiating interventions to ameliorate symptoms and reduce complications. T1DM fulfils the criteria for predictable disease:

1 Genetic markers, specifically HLA class II genes, assist in recognizing individuals at higher risk who are eligible for autoimmune testing and follow-up [26].

2 T1DM has a prolonged prodrome of islet autoimmunity during which cellular and humoral immune markers may be present as early as the perinatal period [27] and remain detectable for months up to several years before onset.

3 Standardized and validated assays have been developed to detect multiple islet autoantibodies with high degrees of diagnostic sensitivity and specificity [28].

4 The extent of β-cell loss and the remaining function may be indirectly evaluated by assessing FPIR and C-peptide levels, for example [29].

Combining these markers provides highly predictive tools that may help to identify individuals at risk and initiate prevention or intervention at an appropriate point in time.

Currently, exogenous insulin injection is the only therapeutic measure that is able to correct hyperglycemia and enables patients with T1DM to live an almost normal life. Nevertheless, complications are common and may develop within 10–12 years after onset even with adequate metabolic control [30,31]. Prediction of T1DM may provide a window of opportunity to initiate prevention programs to reduce or ideally to prevent β-cell destruction. Identifying susceptible individuals prior to the clinical onset may allow an early and symptom-free diagnosis of diabetes and prevent an otherwise acute T1DM. This is crucially important to prevent the life-threatening condition of diabetic ketoacidosis (DKA) [32], which is commonly seen in children <4 years of age and associated with high morbidity and mortality.

Genetic markers

Much of our knowledge about prediction of T1DM has been generated from studies on FDR of patients with T1DM. These studies have revealed that T1DM can be predicted in siblings of patients with diabetes with analysis of HLA genotypes, number of islet autoantibodies and the titer of IA-2Ab [33]. HLA-identical siblings are at greater risk of developing autoantibodies and T1DM than are non-identical siblings.

Almost 50% of T1DM risk among FDR of Caucasian patients with T1DM is attributed to HLA [34]. One or both of the high-risk class II DQ8 and DQ2 haplotypes were found in more than 90% of patients diagnosed before the age of 30. Additionally, nearly 50% of siblings who carry high-risk HLA genes develop islet autoimmunity by the age of 3 years [35]. By contrast, only 15% of children developing T1DM have FDR with the disease. Therefore, screening of the general population is necessary in order to find a majority of children who are susceptible to T1DM. The diagnostic sensitivity of large-scale genetic screening is low because only 10% of subjects with disease-associated HLA genes develop the disease [36]. Nevertheless, several studies such as the BABYDIAB [37], DAISY [38], DiPiS and TEDDY [39], involving genetically susceptible individuals, continue to improve our understanding of the etiopathologic mechanisms of T1DM. Furthermore, the frequencies of DR-DQ alleles within the high-risk haplotypes DR3-DQ2 (*DRB1*03-DQA1*0501-B1*0201*) and DR4-DQ8 (*DRB1*04-DQA1*0301-B1*0302*) vary among different populations [40]. Indeed, T1DM in Asians is associated with HLA haplotypes other than those in Caucasians, indicating that screening strategies may need to be tailored to the population at hand [41]. Longitudinal studies will identify the relationship between HLA variants and the contribution of environmental factors to risk of T1DM. Other non-HLA genetic markers such as *INS-VNTR*, *PTPN22*, *IL2RA* and many others have also been identified [42]. The role of these non-HLA genetic factors in the pathogenesis of islet autoimmunity needs to be clarified, but the relative risk of these genetic factors is shown to be less than that of the HLA genes [42]. HLA and some of the non-HLA genes may be associated with the presence of islet autoantibodies and progression to persistent autoimmunity [43–47]. Combining genetic and autoimmune markers does not improve diagnostic sensitivity of autoantibody testing although the overall positive predictive value may increase.

Autoimmune markers: islet cell autoantibodies

The appearance of diabetes-associated autoantibodies is the first measurable sign of the autoimmune process that eventually leads to T1DM. In 1974, autoantibodies to pancreatic cells were described for the first time [48,49], providing evidence for T1DM being an autoimmune disease. These autoantibodies were named islet cell autoantibodies (ICA) and were detected by immunofluorescence in patients with T1DM. Later, ICA were detected in one patient a year before T1DM onset and in one twin without diabetes who later developed the disease [48], providing the first evidence of an ongoing autoimmune process before the clinical onset of T1DM. ICA are not specific for β-cells and probably represents several different specific autoantigens.

The first autoantigen demonstrated was a Mr64K protein immunoprecipitated by sera from patients with T1DM [50], which was later demonstrated to have glutamic acid decarboxylase (GAD) activity [51], but found not to be a previously recognized isoform of glutamic acid decarboxylase (GAD65) [52]. GAD65 converts glutamic acid to γ-aminobutyric acid (GABA), an inhibitory transmitter in the CNS. GAD65 is primarily present in specific neurons in addition to the pancreatic islet β-cells. Autoantibodies to GAD65 (GAD65Ab) are present in 55–60% of children with T1DM at onset of the disease.

In 1983 autoantibodies to insulin (IAA) were found to be present in patients with diabetes before insulin treatment [53]. This finding suggested that IAA had a role in the autoimmune process of diabetes. IAA are not detected by the immunofluorescence test for ICA. IAA are present in about 35–40% of children with T1DM at onset of the disease.

A third autoantigen, also found to contribute to the ICA reaction, is the insulinoma-associated protein 2 (IA-2), identified in 1996 [54]. IA-2 is a non-functional member of the protein tyrosine phosphatase family. It is localized to the membrane of the secretory vesicles in endocrine and neuronal cells [55]. IA-2 is expressed in both islet α and β-cells, but the function is still unknown because it lacks enzymatic activity. Autoantibodies to IA-2 (IA-2Ab) are directed to the intracellular protein domains [55]. IA-2Ab are present in about 70% of children with T1DM at onset of the disease.

Most recently, autoantibodies to a fourth islet autoantigen, zinc transporter 8 (ZnT8Ab), were reported in patients recently diagnosed with T1DM [56]. This autoantigen is highly β-cell specific. ZnT8 is a protein located in the membrane of the secretory vesicles of the β-cells acting as a zinc ion transporter. Recently, ZnT8Ab have been found in 60–65% of patients with T1DM, compared to <2% in controls [56]. ZnT8Ab are independent markers of islet cell autoimmunity and were detected in otherwise IAA-negative individuals. Furthermore, ZnT8Ab were found to appear and precede disease onset in prospectively studied FDR of patients with T1DM and high-risk individuals from the general population.

Some patients are positive for ICA, but negative for both GAD65Ab and IA-2Ab, suggesting that there are additional, as yet undiscovered, antigens covered by ICA. Some of those patients may have ZnT8Ab. More than 90% of patients with T1DM have previously been reported to have ICA, GAD65Ab, IA-2Ab or IAA at diagnosis. The corresponding number for the general population is about 1% [57]. With the addition of ZnT8Ab to the combined analysis of GAD65Ab, IA-2Ab and IAA, up to 95% of all individuals developing T1DM are positive for at least one autoantibody.

In addition, several other proteins have been proposed as candidate autoantigens (see Chapter 9). Further studies are needed to establish the possible importance of these additional autoantigens for T1DM risk. It is possible that all diabetes-associated autoantibodies have not yet been discovered.

Individuals with clinical T1DM and absence of autoantibodies at diagnosis may still have had an ongoing autoimmune process leading to β-cell destruction. It has been found that 25% of children with T1DM with negative autoantibodies at diagnosis of the disease had autoantibodies present in their cord blood [58].

In genetically predisposed children, detecting islet autoantibodies as early as 3 months of age was associated with high seroconversion rates, where IAA and GAD65Ab often preceded ICA and IA-2Ab appeared last [59,60]. These autoantibodies seem to appear sequentially, with the appearance of additional autoantibodies usually taking place within a year after the detection of the first one [36,61]. While a single autoantibody may be harmless and often represents non-progressive β-cell autoimmunity, the appearance of multiple autoantibodies most often reflects a progressive process [62–66]. The number of detectable autoantibodies is related to the risk of T1DM, both in FDR and in the general population. In studies of family members of patients with T1DM, 60–100% of individuals with three or more autoantibodies develop clinical T1DM over the next 5–6 years and population-based studies indicate that the risk is similar in the general population [67–69]. The autoantibody pattern differs among age groups. It has been suggested that GAD65Ab is a marker of general non-specific autoimmunity, while IA-2Ab and IAA are more specific markers for β-cell death [70]. The recently discovered ZnT8Ab has been reported to appear generally after 3 years of age and thereafter to increase in frequency with age up to adolescence [56].

Titers of the islet autoantibodies need to be taken into consideration for prediction. Higher titers may better predict risk of persistent autoimmunity and T1DM [33,62,64,66], especially in combination with a high-risk HLA genotype [64,71]. Persistent detection of high titer autoantibodies may mirror the intensity of the autoimmune reaction and the rate of progression to clinical diabetes [64].

In studies of the general population, GAD65Ab and IA-2A have been shown to be of equal predictive value as they are in FDR siblings, but for each separate autoantibody a higher cumulative risk was observed among the siblings. Double autoantibody positivity confers a similar cumulative risk among siblings and the general population [69], with additional predictive information provided by the level of the autoantibodies [64]. In one study of school children, the positive predictive value of multiple autoantibodies in the general population was 25–75%, with a sensitivity of 58–100%, not taking HLA genotypes into account [67].

Biochemical (metabolic) markers

Children progressing to T1DM have been shown to have several autoantibodies, high titers of ICA, IA-2Ab and GAD65Ab and decreased FPIR in response to an intravenous glucose tolerance test (IVGTT). A combination of islet autoantibodies and FPIR might therefore potentially predict diabetes [62]. In one study, siblings of children with diabetes were also examined at the time when the index case was diagnosed. Islet autoantibodies alone predicted T1DM in 36%, but when FPIR was taken into account the rate of prediction increased to 56%, with a median observation time of 3.6 years. A young age, a strong humoral response and reduced FPIR seemed to characterize individuals with a progressive process [62]. Additionally, reduced FPIR levels (<50 mU/L) among autoantibody-positive subjects (mainly ICA or IAA) may predict T1DM among FDR by up to 92% over 10 years [29]. C-peptide may predict β-cell functional loss near diagnosis because C-peptide levels may diminish significantly 6 months prior to onset [72]. Therefore, combining these two tests may detect metabolic disturbances and predict the disease prior

to clinical onset, especially when monitored in parallel with autoimmune markers. Children developing diabetes in the DPT-1 study had a gradually deteriorating glucose tolerance with declining 2-hour C-peptide levels after an oral glucose tolerance test (OGTT) [73]. These data were seen over a period of at least 2 years before onset of disease, despite the fact that fasting C-peptide levels remained stable [73]. Moreover, among ICA-positive subjects recruited in the DPT-1 trial, an alternative OGTT index (using area-under-the-curve glucose, 60 and 90-minute glucose, instead of 2-hour glucose) was shown to have better predictive criteria than the standard OGTT, especially when combined with C-peptide measurement from the alternative OGTT [74].

Body mass index (BMI) has also been reported to give additional information in T1DM prediction [75]. It has also been reported that a normal but rising HbA_{1c}, a measure that is proportional to the average blood glucose in the previous 120 days, predicts clinical onset of diabetes in autoantibody-positive children [76].

Other markers

The search for additional biomarkers to predict T1DM is ongoing. Cytokines, chemokines and adhesion molecules are proposed as important inflammatory mediators in the pathogenesis of T1DM (for review see Purohit and She [77]). Antigen-specific T-lymphocytes are also proposed markers for T1DM prediction, but standardized tests to detect these markers have yet to be developed.

Prevention and intervention

Several clinical trials aiming to prevent T1DM have been initiated during the past two decades; some have been completed and others are still ongoing. The general aim of prevention is either to prevent islet autoimmunity from happening or retard the autoimmune destruction of β-cells, or alternatively to preserve remaining β-cell secretory capability before clinical diagnosis. The aim of intervention is to interfere with the natural history of T1DM after the clinical diagnosis. The idea of intervention is to stop or halt the killing of β-cells and preserve β-cell secretory function in patients who have already developed clinical diabetes. Therefore, scientists continue to test new drugs with attempts to halt one or both of these stages. The rationale behind reported prevention and intervention has often been obtained from studies on laboratory animals such as the NOD mouse and BB rat [78–80]. Significant differences exist, however, between these animals and humans; therefore a successful intervention in these animals may not have the exact same effects in humans. Moreover, factors such as the duration of the intervention, the stage of enrolment and drug dosage and safety may influence the outcome. These factors, in addition to the complexity of the etiopathogenesis of T1DM, may explain why little success has been achieved so far in prevention or intervention of T1DM.

All three levels of disease prevention (primary, secondary and tertiary) may be applicable to T1DM.

Primary prevention trials

Primary prevention trials aim to prevent the initiation of autoimmune responses among infants with genetic susceptibility (high-risk HLA genes) or history of T1DM among FDR. Several attempts to prevent T1DM in relatives of patients with the disease have been performed over the years. Such trials are often implemented for longer follow-up durations, especially during the main window of autoimmunity triggering (from birth to 6 years) [81]. Primary prevention trials tend to test the effects of safe, mainly non-antigen-specific agents in relation to environmental risk determinants, of which nutritional factors are important. Results from observational studies encouraged trials that use dietary manipulation to prevent T1DM (Table 59.2).

Non-antigen specific primary prevention trials

Gluten-free diet

A gluten-free diet was given to islet autoantibody-positive children without any significant preventive effect neither on the risk of T1DM nor on the titers of T1DM-associated autoantibodies [82].

Cow's milk

There are several suggestions that cow's milk may be associated with higher risk of T1DM [83]. Dietary manipulation using hydrolyzed casein milk formula provided evidence of preventive effect on the risk of T1DM [84,85]. The TRIGR trial is an international effort involving 17 countries to verify if hydrolyzed casein milk formula can reduce T1DM risk among infants at risk (genetically predisposed infants with history of T1DM among FDR), if used instead of cow's milk. Following a period of 6–8 months' breastfeeding, infants are randomized into either receiving hydrolyzed casein-based or conventional cow's milk formulas. This trial is still ongoing and the results of autoantibody analyses are expected in 2012 and the results of T1DM in 2016 [86].

Vitamin D

Supplementary vitamin D has been suggested to protect against T1DM [87], possibly through the effects on the T lymphocytes and through suppression of cytokine production [88]. Lower concentrations of vitamin D have been reported in blood from children with T1DM, and lower vitamin D levels during pregnancy have been suggested to increase the risk for the child to develop T1DM [88,89]. Vitamin D (cholecalciferol) is currently tested in an ongoing randomized feasibility pilot study in Manitoba, Canada, to test if high doses of vitamin D (2000 IU/day) prevent T1DM among genetically susceptible infants ≤4 weeks of age [90].

Nutritional Intervention to Prevent Diabetes study

Supplementation with cod liver oil, an important source of vitamin D and omega-3 fatty acids, during the first year of life led to reduced risk of T1DM in Norwegian children, but no risk

Table 59.2 Primary prevention trials in T1DM. Adapted from Staeva-Vieira *et al.* [94].

Trial name	Agent	Type of trial	Description	Outcome
Non-antigen-specific trials				
PREVFIN	Gluten-free diet	Pilot	Exclusion of dietary gluten	No effects Ended in 2002
TRIGR	Hydrolized casein formulas vs cow's milk	R/PC, DB	To recruited 2800 type infants with FDR and T1DM Implemented in 17 countries	Ongoing NCT00179777
NIP	Oral omega-3 DHA	R/DB Pilot	Infants <5 months and mothers in third trimister (+ve family history) Follow-up until age of 9 years	Primary results awaited NCT00333554
Vitamin D	Cholecalciferol 2000 IU/day	R/OL Pilot	Genetically susceptible infants up to 4 weeks old (Manitoba, Canada)	Phase 1: Results awaited NCT00141986
Antigen-specific trials				
Pre-POINT	Oral/nasal insulin	R, PC	Genetically predisposed FDR infants (18 months to 7 years) No islet autoimmunity	Recruitment started in Germany and USA in 2010 Duration: 3–18 months

DB, double blinded; DHA, docosahexanoic acid; FDR, First-degree relatives; OL, open label; PC, placebo controlled; R, randomized.
For further details, see clinical trial identifier: http://www.clinicaltrials.gov/ct2/search.

reduction was found with other kinds of vitamin D supplementation, suggesting that omega-3 fatty acids were responsible for the effect [91]. In a recent study it was reported that the intake of omega-3 and omega-6 fatty acids were inversely associated with the development of IAA, GAD65Ab and IA-2Ab in children with genetic risk of T1DM [92].

The Nutritional Intervention to Prevent Diabetes (NIP-Diabetes) is a small pilot study to test a proposed preventive effect of oral docosahexanoic acid (DHA) against islet autoimmunity (trial identifier NCT00333554) [93]. Participants included in this trial were infants ≤5 months of age and expected babies of pregnant mothers in their third trimester (>24 weeks) who were genetically predisposed (DR3 or 4) and had family history of T1DM among FDR. DHA is taken in late pregnancy and early infancy and all those infants are followed up for markers of autoimmunity and T1DM (for review see Staeva-Vieira *et al.* [94]).

Antigen-specific primary prevention trials

Pre-POINT Trial

The primary intervention with oral and/or nasal insulin for prevention of T1DM in infants at high genetic risk to develop diabetes (Pre-POINT) is a trial to identify the dose and the type of insulin that can prevent progression to T1DM. Pre-POINT is an ongoing trial, which has two arms: oral and intranasal. 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).

Secondary prevention trials

Secondary prevention trials are mainly intended for genetically predisposed children in whom autoimmunity has already developed and also for young adults with multiple islet autoantibodies. These trials aim to reduce β-cell killing by autoimmunity and prevent progression to clinical diabetes [95]. Secondary prevention trials may be divided into two groups: non-antigen-specific (Table 59.3) and antigen-specific trials (Table 59.4).

Non-antigen-specific secondary prevention trials

Non-antigen-specific agents were tested in a group of secondary prevention trials including cyclosporine [5,7], BCG vaccine [13], ketotifen [15] and nicotinamide, as in ENDIT [17] and DENIS studies [18]. Although these agents were successful in laboratory animals such as the NOD mice, they failed to show similar benefits in human trials. In regards to immunosuppressive agents, the adverse effects outweighed the temporary benefits observed on indicators of β-cell function [8].

Antigen-specific secondary prevention trials

It is known that an early diabetes diagnosis with mild or no symptoms is associated with good residual capacity of the β-cells to produce insulin and a longer "honeymoon" period [96]. Therefore, it was suggested that insulin therapy in individuals with subclinical diabetes could reduce the β-cell load and be advantageous. Insulin was used in prevention trials as an antigen-specific agent using different routes including parenteral, oral and intranasal insulin. Earlier pilot studies [97,98], which tested parenteral insulin (subcutaneously and intravenously) as prophylaxis among ICA-positive FDR, introduced evidence of delaying disease progression, but the randomized and controlled DPT-1 (parenteral arm) [22] failed to reproduce these results.

It is suggested that insulin given in small doses, either orally or nasally, could induce antigen-specific T-cell tolerance to insulin, which by releasing inhibitory cytokines in the target organ would suppress the autoimmune process against β-cells. This principle

Table 59.3 Non-antigen-specific secondary prevention trials in T1DM. Adapted from Staeva-Vieira *et al.* [94].

Trial name	Agent	Type of trial	Description	Outcome
Cyclosporine	Cyclosporine orally 7.5 mg/kg/day/year	Pilot	FDR with immunologic and metabolic predisposition	Delayed progression, but no prevention
BCG vaccine	BCG vaccine intradermally	Pilot	A group of pilot studies	No effect
Ketotifen	Ketotifen orally	Pilot	ICA +ve subjects with low FPIR	No effect
ENDIT	Nicotinamide Orally 1.2 g/m^2/day	R/PC, DB	ICA +ve FDR of young diabetes onset (<20 years)	No effect
DENIS	Nicotinamide Orally 1.2 g/m^2/day	R, PC	ICA +ve FDR at risk of type 1 diabetes within 3 years	No effect
Nicotinamide combinations	Nicotinamide orally + intensive insulin or cyclosporine or vitamin E	Pilot	Various combinations used with nicotinamide	No effect

BCG, Bacille Calmette–Guérin; DB, double blinded; FDR, first-degree relatives; FPIR, first-phase insulin release; ICA, islet cell autoantibodies; PC, placebo controlled; R, randomized.
For further details, see clinical trial identifier: http://www.clinicaltrials.gov/ct2/search.

Table 59.4 Antigen-specific secondary prevention trials in T1DM. Adapted from Staeva-Vieira *et al.* [94].

Trial name	Agent	Type of trial	Description	Outcome
DPT-1	Parenteral human ultralente insulin s.c. 0.25 U/kg/day + 1 annual infusion	RC/ET	Large multicenter study: FDR with >50% disease risk in 5 years	No effect
DPT-1	Oral insulin 7.5 mg/day	R/PC, DB, ET	Large multicenter study: FDR with 26–50% disease risk in 5 years Follow-up with OGTT for 6 months	No effect (subanalysis showed delay of onset in subjects with IAA >80 U/mL) Phase III is ongoing
DIPP	Intranasal insulin	R/DB/PC PA, ET	Genetically predisposed infants. Tested for ICA, IAA, GAD65Ab and IA-2Ab and IVGTT	No effect after follow-up to 15 years of age
INIT I	Intranasal insulin	OL	Tested effect on autoimmunity among children and young adults	No effect
INIT II	Intranasal insulin 1.6 and 16 mg	R/DB/PC PA, ET	Children and young adults at risk of type 1 diabetes Followed for β-cell function and AAb & OGTT/6 months	Ongoing NCT00336674

AAb, autoantibodies; DB, double blinded; ET, efficacy trial; FDR, first-degree relative; GAD 65Ab, glutamine acid decarboxylase autoantibodies; IAA, insulin autoantibody; IA-2Ab, autoantibody to insulinoma-associated antigen 2; ICA, islet cell autoantibodies; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test; OL, open label; PA, parallel assignment; PC, placebo controlled; R, randomized; RC, randomized controlled.
For further details, see clinical trial identifier: http://www.clinicaltrials.gov/ct2/search

has previously been tried in various other autoimmune diseases, with small or inconsistent beneficial effects. The theory is that peptides from orally ingested antigens encounter the lymphoid tissue in the mucosa, which serves to protect the host from reacting against ingested proteins.

Oral insulin

The oral arm of the DPT-1 trial examined a preventive effect of oral insulin (7.5 mg/day) [23]. This placebo-controlled study tested 372 high-risk first and second-degree relatives who were positive for ICA and IAA, but with normal FPIR on IVGTT and glucose tolerance on OGTT. Although the overall results of the DPT-1 showed no significant effect, subanalysis of these data showed a benefit in subjects with IAA >80 U/mL, in whom diabetes was delayed by 4–5 years [23]. This indicates that oral insulin may have a protective effect when given to individuals at risk and having high titers of IAA. No serious study-related adverse events were recorded with oral insulin and the levels of

IAA in the patients did not increase with the administration of oral insulin [99]. Further studies of orally administered insulin in subjects with high titers of IAA are ongoing such as the ongoing repeat of the oral arm of DPT-1 (NCT00419562).

Nasal insulin

Prophylactic administration of insulin nasally has shown to prevent T1DM in NOD mice. In humans, insulin has also been used nasally to induce autoimmune tolerance in prevention trials such as the Intranasal Insulin Trial (INIT) phases I and II and Type 1 Diabetes Prediction and Prevention trial (DIPP). The INIT is a double-blinded cross-over pilot study that recruited T1DM FDR in Australia. INIT-I was completed in 2004 with no significant effect on β-cell function but it showed some indications of mucosal tolerance to insulin [24]. INIT-II (NCT00336674) is an ongoing randomized double-blinded placebo-controlled trial using nasal insulin (1.6 or 16 mg) that aims to assess the effects of nasal insulin on islet autoimmunity. The DIPP study of Finland is a double-blinded prevention trial that used nasal insulin in children with genetic risk and positive ICA and IAA. In 224 children short-acting insulin or placebo was administered intranasally once a day, but no protective effect was seen [25].

In animal studies, oral insulin has shown various results in protecting from T1DM. In BB rats, oral insulin failed to prevent the disease and actually seemed to accelerate the autoimmune process [100,101]. In contrast, studies in the NOD mouse did reveal a protective effect of the drug [102]. Taken together, there is limited evidence that preclinical studies in the NOD mouse and the BB rat can be immediately translated into successful trials in humans.

Intervention (tertiary prevention) trials

Tertiary prevention or intervention trials mainly recruit patients with new-onset T1DM, using interventions intended to preserve β-cells and also to maintain their regeneration [103]. In these studies researchers are trying to suppress or modulate the immune response in order to stop the autoimmune process, which leads to β-cell death and eventually T1DM, or to reduce the β-cell stress, for example by early-introduced insulin therapy. An intensive insulin regimen in patients with new-onset T1DM is proposed to preserve the remaining β-cells and enhance their functionality [104]. Intensive insulin treatment should therefore be used as the basic therapy for all newly diagnosed patients with T1DM who are randomized into intervention trials.

Unlike prevention trials, tertiary interventions mainly test less safe agents over shorter duration of time on subjects who have already developed clinical diabetes. The intervention drug has typically been evaluated in animal studies, and then assessed in small pilot studies to ensure an acceptable route of administration and to verify a safety profile. Intervention trials commonly consider C-peptides as the main outcome for β-cell function, which may reflect the extent of β-cell preservation. Certain trials test combination therapies in which various drug modalities with different modes of action are used in pubertal patients with new-onset T1DM (commonly two drugs in each trial).

Therapeutic agents used in tertiary interventions may also be classified into non-antigen-specific (Table 59.5) or antigen-specific (Table 59.6).

Non-antigen-specific intervention trials

In this set of trials non-antigen-specific drugs are used including immunosuppressive or immunomodulatory agents (Table 59.5). Systemic immunosuppressive drugs such as cyclosporine [7,105] and azathioprine [106] induced remission with signs of improved β-cell function in patients with new-onset T1DM. In spite of that, serious side effects limited prolonged clinical use.

Nicotinamide

Nicotinamide was also used among patients with new-onset T1DM in a group of intervention trials [107–109]. Some of these trials proposed a beneficial effect of nicotinamide reflected in improved C-peptide levels among intervention groups compared with controls [109]. This may indicate that nicotinamide possesses certain therapeutic effects related to β-cell function after onset, but not during the autoimmune prodrome, because prevention trials did not prove a beneficial effect of nicotinamide among high-risk individuals [17,18].

Other agents such as the BCG vaccine [14,110] and diazoxide [111] failed to produce sustained beneficial effect on preserving β-cell function.

Anti-CD3 monoclonal antibodies

Non-specific systemic immunotherapy to modulate T cells has also been attempted with some promising outcomes among this group of drugs. Anti-CD3 monoclonal antibodies (anti-CD3 MoAb), such as teplizumab, have shown some positive effects in postponing the autoimmune destruction of β-cells with preservation of their functionality and enhancement of endogenous insulin [112–114]. Humanized anti-CD3 MoAb were used in order to avoid depleting T-lymphocyte populations. Two variants of these antibodies, mutated Fc region (hOKT3γ1(Ala-Ala) and glycosylated Fc region ChAglyCD3(TRX4)) were used in two already completed trials [112,113]. These trials showed that anti-CD3 MoAb were able to enhance C-peptide levels and improve clinical variables for up to 2 years but they did not induce sustained tolerance of β-cell autoantigens [114]. This effect appears to be transient because the drug only delayed the decline in C-peptide concentration. Despite remission and metabolic enhancement, there were serious adverse effects including bone marrow suppression with cytokine release syndrome or subsequent serious infections or reactivation of Epstein–Barr virus infection with this treatment [113]. Nevertheless, the obtained results encouraged initiating phase II of the anti-CD3 trial (NCT00378508) and planning a third phase.

Antithymocyte globulin

Antithymocyte globulin (ATG) was found to induce short-term benefits in laboratory animals such as the NOD mice, primarily through inducing immunoregulation rather than depleting T-lymphocytes [115]. In humans, ATG is thought to decrease

Table 59.5 Non-antigen-specific intervention trials in T1DM. Adapted from Staeva-Vieira *et al.* [94].

Trial name	Agent	Type of trial	Description	Outcome
Cyclosporine	Cyclosporine-A	R, PC	A group of trials	Remission but with serious side effects
ChAgly CD3	Humanized non-mitogenic CD3 monoclonal Ab 8 mg/day IV/6 days	R, DB, PC, PA, ET	Starts at diagnosis of patients with new onset T1DM 12–45 years	Completed, results awaited NCT00627146
Teplizumab	Anti-CD3 monoclonal Ab hOKT3γ1 (Ala-Ala)	R, DB, PC, PA, ET	Phase II: Single 14 course for 4–12 months after diagnosis of new-onset T1DM, to test effects on insulin secretion	Ongoing NCT00378508
ATG	Antithymocyte globulin	R, SB, PA, ET	Test the effect of ATG plus intensive insulin versus intensive insulin therapy only	Ongoing NCT00190502
STRAT	Anti-thymocyte globulin (ATG) Daily 4-day escalating dose	R, DB, PC, PA, ET	Phase II: Given within 12 weeks of diagnosis to assess effects on insulin secretion (C-peptide) over 12 months	Ongoing ITN028AI
Rituximab	Anti-CD20 monoclonal Ab weekly IV/4 weeks	R, DB, PC, PA, Safety/ET	Phases II & III: 8–45 years patients with T1DM, followed every 3 months/2 years (C-peptide, insulin and HbA_{1c})	Results awaited NCT00279305
Abatacept (CTLA-4)	Cytotoxic T-lymphocyte antigen-4, IV	R, DB, PC, PA, ET	The effect of CTLA-4 on insulin production (C-peptide secretion) over 2 years	Ongoing, not recruiting NCT00505375
HrIFN-α	Oral human recombinant interferon α	R, DB, PC, PA	Phase II: test the effect on counter-regulatory anti-inflammatory cytokines	Ongoing, not recruiting NCT00005665
Anakinra, Kineret®	IL-1 receptor antagonist	E, OL	Whether Anakinra protects β-cells by blocking the IL-1β receptor in children with T1DM	Ongoing NCT00645840
Anakinra, Kineret®	IL-1 receptor antagonist	R, DB, PC, PA, ET	The effect of IL-1β receptor antagonism on insulin secretion in young adults with T1DM	Ongoing NCT00711503

ATG, antithymocyte globulin; DB, double blinded; E, exploratory; ET, efficacy trial; IL, interleukin; OL, open label; PA, parallel assignment; PC, placebo controlled; R, randomized; SB, single blinded.
For further details, see clinical trial identifier: http://www.clinicaltrials.gov/ct2/search.

Table 59.6 Antigen-specific intervention trials in T1DM. Adapted from Staeva-Vieira *et al.* [94].

Trial name	Agent	Type of trial	Description	Outcome
DiaPep77 (HSP60)	Subcutaneous peptide heat shock protein 60	R, PC, DB, PA, ET	An immunomodulatory peptide proposed to protect internal production of insulin through halting β-cell killing	Preserved β-cell over 12–18 months in adults. No similar effect in children Phase III (DIA-AID) is ongoing NCT00615264
Alum-GAD (Diamyd®)	Alhydrogel-formulated GAD65 20 μg s.c.	R, PC, DB, PA, ET	Immuno-modulation effect in LADA (Phase IIa) and 10–18 years old with new-onset T1DM (Phase IIb)	Preserved β-cell and C-peptide levels in patients with <6 months onset Phase III ongoing NCT00723411
NBI-6024	Altered peptide ligand insulin B:9–23 vaccine s.c.	R, DB, PC, PA, ET	Multinational trial used a genetically engineered peptide in adolescents (10–17 years) and adults (18–35 years) with new onset T1DM	Completed NCT00873561
BHT-3021	Pro-insulin-based DNA vaccine weekly i.m. inj./12 weeks	R, DB, PC, OL, CO	Assesses safety in ≥18-year-old patients with T1DM: 12 months blinded treatment, 12 months cross-over and 12 months follow-up	Ongoing NCT00453375

CO, cross-over; DB, double blinded; ET, efficacy trial; i.m. inj., intramuscular injection; LADA, latent autoimmune diabetes of adults; OL, open label; PA, parallel assignment; PC, placebo controlled; R, randomized; RC, randomized controlled; s.c subcutaneous.
For further details, see clinical trial identifier: http://www.clinicaltrials.gov/ct2/search.

insulin requirement in patients with new-onset T1DM; however, serious adverse effects such as transient thrombocytopenia are major drawbacks to be considered. Currently, polyclonal anti-T-lymphocyte globulin is being tested in an ongoing randomized placebo control trial that aims to prevent progression of autoimmune β-cell destruction in patients with new-onset T1DM (NCT00190502).

Anti-CD20 monoclonal antibodies

Anti-CD20 MoAbs are potential non-specific immunoregulatory agents. These monoclonal antibodies induced regulatory B lymphocytes in humanized mice [116]. It is proposed that monoclonal antibodies exert an immunoregulatory effect through reducing B lymphocytes and decreasing their antigen-presentation abilities, cytokine release and antibody production [116]. In humans, anti-CD20 MoAb (rituximab) is currently used in a randomized double-blinded placebo-controlled trial (NCT00279305, phases II and III) to examines whether rituximab can preserve residual insulin and halt β-cell function loss.

Abatacept

The cytotoxic T-lymphocyte antigen 4 (CTLA-4) is thought to be involved in modulating immune responses through inducing co-stimulatory signals, which are important for T-lymphocyte activation [117]. CTLA4 immunoglobulin (CTLA4-Ig) is proposed to regulate, but not delete, T-lymphocytes through inhibiting their stimulatory activation pathway [118], and is therefore considered relatively safer than other immunosuppressive agents. CTLA4-Ig (abatacept) was recently used in a randomized placebo controlled trial to assess the effects of CTLA4-Ig on progression of new-onset T1DM in patients aged 6–45 years (NCT00505375).

Human recombinant interferon α

Several other non-antigen specific interventions are currently ongoing. A randomized double-blinded trial assessing whether orally ingested human recombinant interferon α (hrIFN-α) may prolong the "honeymoon" period in patients with newly diagnosed T1DM aged 3–25 years (NCT00005665) examines whether oral hrIFN-α enhances counter-regulatory anti-inflammatory cytokines such as interleukin-4 (IL-4), IL-10 and peripheral IFN-α, thereby maintaining residual living β-cells.

Interleukin-1 receptor antagonist

Two trials were recently launched to test the anti-inflammatory effect of IL-1 receptor antagonist (Anakinra, Kineret®) on maintaining β-cell function and preserving insulin secretion in children (NCT00645840) and young adults (NCT00711503). Based on results from preclinical T1DM studies and studies in rheumatoid arthritis [119], these trials propose that immune regulation in T1DM may be obtained through blocking the IL-1β receptor, which has an important role in β-cell killing.

Antigen-specific intervention trials

Specific pancreatic antigens have been examined as safe agents to modulate the autoimmune response through inducing immunologic tolerance towards islet antigens. In experimental animals, several antigen-specific molecules are able to induce immunologic tolerance and protection against T1DM [120] using a whole islet protein, peptides taken from islet antigen, or DNA-based vaccines [121]. In humans, only few potential antigen-specific agents may have promising results such as peptide heat shock protein 60 (DiaPep277) and alum-formulated GAD65. The induction of immunologic tolerance using antigen-specific agents is thought to be achieved through the stimulation of antigen-specific regulatory T lymphocyte (T_{reg}); however, standardized specific tools for detecting and measuring these T_{reg} cells are not yet available. A group of intervention trials is currently under way to examine the effect of certain antigen-specific drugs in new-onset T1DM (Table 59.6).

DiaPep277

The humanized DiaPep277 was found to provide protection for β-cells and enhance the levels of C-peptide in a group of trials [122–124]. DiaPep277 was reported to possess a high safety profile; however, the deterioration in β-cell function over an 18-month duration after initiating the intervention was more apparent in children (≤15 years) than in adults [123,124]. A trial with similar design involving newly diagnosed children found no similar beneficial effect on β-cell function and C-peptide levels [125]. DiaPep277 is currently tested in a randomized double-blinded placebo-controlled trial involving patients with new-onset T1DM aged 16–45 years (NCT00615264 phase III).

Alum-GAD65

Another way of trying to induce tolerance to an antigen is to give the antigen subcutaneously in small and repeated doses together with aluminium hydroxide. This adjuvant is commonly used in vaccines. Aluminium salts preferentially induce a humoral (Th2) rather than cellular (Th1) immune response. Because the autoimmune process leading to T1DM is thought to be a mainly cellular (Th1) immune response, aluminium salts could steer the process against a humoral (Th2) response and minimize the likelihood of exacerbating cell-mediated β-cell destruction. This concept is used in the trials of vaccination with Alhydrogel-formulated GAD65 (Diamyd®).

A dose-finding placebo-controlled Phase IIa safety study in 47 patients with latent autoimmune diabetes in adults (LADA) indicated that 20 μg Diamyd reduced β-cell destruction without any serious adverse events. In Phase IIb clinical trial, the efficacy and safety of Diamyd were investigated in children 10–18 years of age, recently diagnosed with T1DM [126]. A total of 70 GAD65 antibody-positive children with a recent diagnosis (<18 months duration) of T1DM were included in the study. All had remaining β-cell capacity measured by C-peptide levels. A total of 35 children received two doses of 20 μg Diamyd and 35 children received placebo. C-peptide levels were measured after mixed meal tolerance tests 3, 9, 15, 21 and 30 months after the first drug injection. Significant differences between groups were found after mixed meal stimulation and fasting C-peptide was different at 30 months

only. Furthermore, in a subgroup of children who had been diagnosed with diabetes no longer than 6 months at study-start, the fasting C-peptide levels declined less in children who had received Diamyd compared with the levels in children who received placebo. This indicates that Diamyd may prevent further β-cell destruction in recently diagnosed children, without any serious adverse events. Thus, the study has provided support for the clinical safety of Diamyd and statistically significant and clinically relevant positive effect on the preservation of β-cell function in children diagnosed within 6 months before study start [126]. A Phase III randomized double-blinded placebo-controlled multicenter study is ongoing to evaluate the efficacy and safety of Diamyd further, with a larger number of children diagnosed no longer than 3 months before inclusion in the study.

Studies of the NOD mice indicated that GAD65 prevents the autoimmune destruction of the pancreatic β-cells and T1DM [127–132].

Insulin peptides

It has been observed in animal studies that intermittent immunization with insulin or insulin-β chain coupled with incomplete Freund adjuvant may prevent T1DM progression in NOD mice [133]. Another approach used altered peptide ligand, based on the immunodominant T1DM insulin-β chain:9-23 vaccine (NBI-6024), which was tested in humans based on earlier results from experimental animals [134,135]. This region of insulin-β chain has been shown as a specific T-lymphocyte target antigen in the NOD mouse [134]. A genetically engineered vaccine was prepared based on this peptide region and administered subcutaneously to adolescents and adults with a recent history of T1DM. The Phase I trial showed that NBI-6024 was able to shift the IFN-γ-producing T-helper (Th1) lymphocyte into the Th2 regulatory lymphocyte [135]; however, no preventive effect or metabolic control was observed with this NBI-6024 (NCT00873561).

Pro-insulin-based DNA vaccine (BHT-3021) is an antigen-specific drug which is currently being tested in adults older than 18 years with new-onset T1DM. This randomized blinded placebo-controlled trial is a multicenter study that will test the safety of this drug (NCT00453375).

Combination therapies

The search for a single drug that can modulate the natural history of T1DM continues; however, it is not yet clear to what extent a single agent may be able to modify or retard the progression of T1DM. Therefore, combinations of drugs have also been suggested for patients with new-onset diabetes.

The current direction of research is to combine two immunomodulatory agents: non-specific antibody-based immunotherapy such as anti-CD3 MoAb and specific antigen-based immunoregulatory agent such as proinsulin peptide [136]. This combination may permit reduction of the dose or the duration of one or both drugs to maximize benefits or minimize adverse effects. Bresson and von Herrath suggested two main goals for combination therapy of T1DM [121]. First, ensure rapid interruption of the aggressive autoimmune response with minimum adverse effects. Second, enhance β-cell regeneration to the level of sustaining normal glucose levels independent of exogenous insulin therapy [121].

Among combination therapies aiming at immunoregulation are IL-2 (proleukin) and sirolimus (rapamycin), which are being combined and used in an ongoing open-label uncontrolled safety trial. Participants are adults, 18–45 years old, who were diagnosed with T1DM within 4 years (NCT00525889). This trial was based on results obtained in NOD mice when this therapy combination induced sustained β-cell protection and prevented T1DM progression after omitting the drugs [137]. The synergetic effects of these drugs are thought to be through enhancing shifting Th1 lymphocyte into the protective Th2 regulatory lymphocyte.

A second proposed combination involves anti-CD3 and a proinsulin peptide (B24–C36) nasally. This combination was found to induce proinsulin-specific T_{reg} cells and enhance remission in two experimental animals more than the effect of each single drug alone [136].

Alternatively, an ongoing randomized double-blinded placebo-controlled trial tests two immunosuppressive agents, mycophenolate mofetil (MMF/CellCept) and the anti-IL2 receptor monoclonal antibody, daclizumab (DZB/Zenapax) (NCT00100178). This trial will assess whether this combination preserves the remaining viable β-cells and enhances their regeneration in subjects 8–45 years of age who were diagnosed within a 3-month-period. (For further details on prevention and intervention trials see http://www.ClinicalTrials.gov.)

Future directions

Future drug therapy of T1DM will depend on the success of ongoing and planned intervention trials. Since 1976, patients with newly diagnosed T1DM have been given immunosuppressive agents but none of these have preserved β-cell function. Transient effects have been reported but the benefits have been limited because they had to continue the insulin replacement therapy. Immunomodulation alone, or possibly combined with immunosuppressive therapy, seems to be promising in reducing the loss of C-peptides after diagnosis. It is often questioned whether immunomodulation with a single autoantigen is sufficient. Provided the treatment is safe, it cannot be excluded that the simultaneous administration of GAD65, IA-2 and proinsulin may be more efficacious than GAD65 alone.

The route of administration needs further exploration. Oral insulin has already been tested and it would be interesting to test the efficacy of oral GAD65. Furthermore, it would be interesting to test alum-GAD65 together with alum-formulated proinsulin. Studies of the function of the human immune system lags behind that of the mouse and rat. Safe immune tolerance trials may provide a novel approach to dissect the mechanisms by which the human immune system responds to immune therapy with autoantigens.

Since 2001, TrialNet, an international network of clinical research groups supported by the National Institutes of Health, has established an infrastructure for trials for predicting and preventing T1DM. Within this network, relatives of patients with T1DM will be screened for disease risk and then either followed or randomized into clinical trials. Individuals developing multiple autoantibodies will be eligible for ongoing prevention studies. Networks such as TrialNet will enable different investigators and clinics to cooperate in the attempts to predict and prevent T1DM.

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