Chapter 49
Prevention of Type 1 Diabetes Mellitus

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Introduction

Type 1 diabetes (T1D) results from the autoimmune destruction of insulin-producing beta cells in the pancreas. Genetic, metabolic, and environmental factors act together to precipitate the onset of the disease. The excess mortality associated with complications of T1D and the increasing incidence of childhood T1D emphasize the importance of therapeutic strategies to prevent this chronic metabolic disorder.

Clinical T1D represents the end stage of a process resulting from the progressive beta cell destruction following an asymptomatic period that may last for years. This knowledge, together with recent advances in the ability to identify individuals at increased risk for clinical disease, has paved the way for trials aimed at preventing or delaying the clinical onset of T1D. Individuals at risk for T1D can be identified by a positive family history, or by genetic, immunological, or metabolic markers. These markers can be combined to achieve a higher positive predictive value for T1D and to identify those individuals to be selected for intervention trials.

T1D is one of the most widespread chronic disease of childhood affecting children, adolescents, and young adults. In 1985 people with diabetes (all types included) were 30 millions worldwide, in 1995 135 million, and in 2001 approximately 177 million.[1]

The global incidence of T1D in children and adolescents is rising with an estimated overall annual increase of approximately 3%. The increase in incidence of T1D has been shown in countries having both high- and low-prevalence figures, with an indication of a steeper increase in some of the low-prevalence countries.[2] Several European studies have suggested that, in relative terms, the increase is more pronounced in young children. Although T1D usually accounts for only a minority of the total burden of diabetes in a population, it is the predominant form of the disease in younger age groups in most developed countries.

T1D accounts for about 10% of all cases of diabetes, occurs most commonly in people of European descent, and affects 2 million people in Europe and North America. The lowest incidence has been found in Asia and Oceania, the highest in Europe. In particular for western Europe an increase of cases of T1D of 18.3% from 1994 to 2000 has been observed.[3] There is also a marked geographic variation in incidence, with a child in Finland being about 400 times more likely than a child in Venezuela to acquire the disease. The current global increase in incidence of 3% per year is well reported, and it is predicted that the incidence of T1D will be 40% higher in 2010 than in 1998.[4] This rapid rise strongly suggests a promoting effect of the environment on susceptibility genes in the evolving epidemiology of T1D.

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L. Poretsky (ed.), *Principles of Diabetes Mellitus*, DOI 10.1007/978-0-387-09841-8_49,

Pathogenesis of T1D: An Update in View of Defining Preventive Tools

There are three main categories of factors involved in the pathogenesis of T1D. These are including genetic, immunological, and environmental factors. Genetic studies have been completed in families with multiple members affected by this disease and in monozygotic twins. These studies indicate that in T1D the genetic factors are highly relevant but complex and cannot be classified within a specific model of inheritance.[5]

Like other organ-specific autoimmune diseases, T1D has human leukocyte antigen (HLA) associations. The HLA complex on chromosome 6 comprises the first gene shown to be associated with the disease which is considered to contribute about half of the familial basis of T1D. Two combinations of HLA haplotypes are of particular importance. They are DR4-DQ8 and DR3-DQ2 which are present in 90% of children with T1D.[6] A third haplotype, DR15-DQ6, is found in less than 1% of children with T1D, compared with more than 20% of the general population and is considered to be protective. The genotype combining the two susceptibility haplotypes (DR4-DQ8/DR3-DQ2) contributes the greatest risk of the disease and is most common in children in whom the disease develops very early in life. First-degree relatives of these children are themselves at greater risk of T1D than are the relatives of children in whom the disease develops later.

Candidate gene studies also identified the insulin gene on chromosome 11 as the second most important genetic susceptibility factor, contributing 10% of the genetic susceptibility to T1D.[7] Shorter forms of a variable number tandem repeat in the insulin promoter are associated with susceptibility to the disease, whereas longer forms are associated with protection. Demonstration of increased expression of insulin in the thymus of people with protective repeats suggesting more efficient deletion of insulin-specific T cells provides an attractive potential mechanism for the role of the insulin gene in T1D. Over the last decade, whole genome screens have indicated that there are at least 15 other loci associated with T1D and of those another two genes associated with T-cell activation have been identified. An allele of the gene acting as a negative regulator of T-cell activation, cytotoxic T-lymphocyte antigen 4 (CTLA-4), found on chromosome 2q33, is considered to be the third susceptibility gene for T1D and has been associated with increased levels of soluble CTLA-4 and the frequency of regulatory T cells.[8] A variant of PTPN22, the gene encoding lymphoid phosphatase (LYP), also a suppressor of T-cell activation, has been deemed the fourth susceptibility gene.[9] The observation that the four most important susceptibility genes for T1D can all be represented on a single diagram of antigen presentation to T cells emphasizes the potential importance of current therapeutic strategies targeting this interaction.

Genetic studies have highlighted the importance of large, well-characterized populations in the identification of susceptibility genes for T1D. Recruitment of increasingly large populations of patients with T1D and their families is required to provide statistically powerful cohorts to identify other disease-associated genes. Some genes have a relatively minor individual impact on susceptibility to disease but could nevertheless provide more clues to future preventive therapies.

The presence of autoantibodies to beta cells is the hallmark of T1D. Abnormal activation of the T-cell-mediated immune system in susceptible individuals leads to an inflammatory response within the islets as well as to a humoral response with production of antibodies to beta cell antigens. Islet cell antibodies (ICA) were the first described, followed by more specific autoantibodies to insulin (IAA), glutamic acid decarboxylase (GAD) and the protein tyrosine phosphatase (IA-2), all of which can be easily detected by sensitive radioimmunoassay and are now measured[10] to identify subjects at risk of developing T1D.[11] These autoantibodies are common in both childhood- and adult-onset T1D with many subjects being positive for multiple autoantibodies. The type of immune response is age dependent, but seroconversion to multiple autoantibody positivity usually occurs tightly clustered in time and is associated with genetic risk.

The presence of one or more type of antibody can precede the clinical onset of T1D by years or even decades. These autoantibodies are usually persistent, although a small group of individuals may revert back to being seronegative without progressing to clinical diabetes.[12] The presence and persistence of positivity to multiple antibodies increases the likelihood of progression to clinical disease.

Continuing destruction of beta cells leads to a progressive reduction of insulin secretory reserve and loss of first-phase insulin secretion in response to an intravenous glucose tolerance test, followed by clinical diabetes when insulin secretion falls below a critical amount, and finally, to a state of absolute insulin deficiency.

Supportive evidence for the autoimmune pathogenesis of T1D comes from data showing susceptibility of individuals at risk for T1D to other autoimmune conditions including Hashimoto's thyroiditis, Graves' disease, Addison's disease, coeliac disease, myasthenia gravis, and vitiligo.[13]

Regarding the role of environmental factors, it should be underlined that the increase in incidence of T1D is too rapid to be caused by alterations in the genetic background and is likely to be the result of environmental changes. This is confirmed by recent experiments showing that the increase in T1D has been accompanied by a concomitant widening of the HLA risk profile, which suggests increased environmental pressure on susceptible HLA genotypes. The environmental factors in T1D are difficult to study because the variety of the environmental conditions as well as the possible multiple interactions between putative factors.

What Are the Environmental Factors?

Certain viral infections may play a role in the pathogenesis of human T1D. *Congenital rubella* is the classical example of virus-induced diabetes in human beings, but effective immunization programs have eliminated congenital rubella in most western countries. Currently, the main candidate for a viral trigger of human diabetes are members of the group of *Enterovirus*.[14] They are small non-enveloped RNA viruses, which belong to the Picornavirus family. They consist of more than 60 different serotypes, with the Polioviruses being their best-known representatives. Enterovirus infections are frequent among children and adolescents causing aseptic meningitis, myocarditis, rash, hand-foot-and-mouth disease, herpangina, paralysis, respiratory infections, and severe systemic infections in newborn infants. Most infections, however, are subclinical or manifest with mild respiratory symptoms. The primary replication of the virus occurs in the lymphoid tissues of the pharynx and small intestine, and during the following viremic phase the virus can spread to various organs including the beta cells.

Theoretically, *Enterovirus* could cause beta-cell damage by two main mechanisms. They may infect beta cells and destroy them directly or they may induce an autoimmune response against beta cells.[15] Direct virus-induced damage has been supported by studies showing that *Enterovirus* are present in beta cells in patients who have died from severe systemic *Enterovirus* infection and that the islet cells of these patients are damaged. *Enterovirus* can also infect and damage beta cells in vitro and induce the expression of interferon-alpha and HLA-class I molecules in beta cells, thus mimicking the situation observed in the pancreas of patients affected by T1D. In addition, *Enterovirus* infections may have interactions with other risk factors increasing the immune response to dietary antigens as they replicate in gut-associated lymphoid tissues.[16]

The first reports connecting *Enterovirus* infections to T1D were published more than 30 years ago showing that the seasonal variation in the onset of T1D follows that of *Enterovirus* infections.[17] At the same time antibodies against *Coxsackievirus B* serotypes were found to be more frequent in patients with newly diagnosed T1D than in control subjects.[18] *Enterovirus* have also been isolated from patients with newly diagnosed T1D. In one case report *Coxsackievirus B4* was isolated from the pancreas of a child who had died from diabetic ketoacidosis, and this virus caused diabetes when transferred to a susceptible mouse strain. The beta cells of diabetic patients also express interferon-alpha, a cytokine that is induced during viral infections, suggesting the presence of some virus in the beta cells. Prospective studies are particularly valuable in the evaluation of viral triggers because they cover all stages of the beta-cell damaging process.

Enterovirus are not the only viruses that have been connected to the pathogenesis of T1D. *Mumps*, *measles, cytomegalovirus*, and *retroviruses* also have been found to be associated with T1D, but the evidence is less convincing than that for *Enterovirus*.

The Role of Cow's Milk

There is evidence that cow's milk proteins can act as triggers for the autoimmune process of beta cell destruction based on studies indicating bottle feeding as triggering factor for an autoimmune response to beta cells.

There are several arguments for the milk hypothesis in T1D including the following (reviewed in ref.[19]):

Epidemiological studies show increased risk for T1D if the breast-feeding period is short and cow's milk is introduced before 3–4 months of age.
Skim milk powder can be "diabetogenic" in diabetes-prone BB rats.
Patients with T1D have increased levels of antibodies against cow's milk constituents.
Milk albumin and beta casein have some structural similarity to the islet autoantigen ICA69 and GLUT2, respectively.

A number of hypotheses have been postulated to explain the pathogenic role of cow's milk. One of the most convincing one is that immature gut mucosa allows the passage of high molecular weight, potentially antigenic proteins which share some molecular mimicry with pancreatic beta cells.[20] Among diabetogenic proteins in cow's milk, beta casein, beta lactoglobulin, and albumin have been implicated as sources of potential antigens.

Casein represents the major protein in cow's milk. Human and bovine beta casein are approximately 70% homologous and 30% identical. There are several reasons why it is thought that beta casein is a good candidate to explain the observed association between cow's milk consumption and T1D:[21] (a) it has several structural differences from the homologous human protein; (b) casein is probably the milk fraction promoting diabetes in the NOD mouse, since a protein-free diet prevents the disease while a diet containing casein as the sole source of protein produces diabetes in the same animals; (c) several sequence homologies exist between bovine beta casein and beta cell autoantigens; (d) specific cellular and humoral immune responses toward bovine beta casein are detectable in most T1D patients at the time of diagnosis,[22] highly suggestive that this protein may participate in the immune events triggering the disease; (e) casein hydrolysate was shown to be nondiabetogenic in the BB rat and NOD mouse models; therefore, it was thought that this dietary intervention might be beneficial in humans as well for disease prevention.

The rationale behind the use of cow's milk hydrolysate for primary prevention of T1D is based on several epidemiological and in vitro studies indicating that intact cow's milk, if given before 3 months of age, may induce an immune response toward beta cells.

The Role of Vitamin D Deficiency

Several epidemiological studies have described an intriguing correlation between geographical latitude and the incidence of T1D and an inverse correlation between monthly hours of sunshine and the incidence of diabetes.[23] A seasonal pattern of disease onset has also been described for T1D, once again suggesting an inverse correlation between sunlight and the disease.[24] Vitamin D is an obvious candidate as a mediator of this sunshine effect.

Dietary vitamin D supplementation is often recommended in pregnant women and in children to prevent vitamin D deficiency. Cod liver oil taken during the first year of life reportedly reduced the risk of childhood-onset T1D, and a multicenter case–control study also showed an association between vitamin D supplementation in infancy and a decreased risk of T1D.[25] A further study found that an intake of 2000 IU of vitamin D during the first year of life diminished the risk of developing T1D and showed that the incidence of childhood diabetes was three times higher in subjects with suspected rickets.[26,27] It remains to be determined whether these observations are the result of supplementation of vitamin D to supraphysiological levels or are simply the result of the prevention of vitamin D deficiency. Observations in animal models suggest the latter, since regular supplements of vitamin D in neonatal and early life offered no protection against T1D in non-obese diabetic (NOD) mice or in BB rats, whereas the prevalence of diabetes is doubled in NOD mice rendered vitamin D deficient in early life.[28] The results of genetic studies investigating a possible relationship between VDR polymorphisms and T1D are inconsistent: a clear correlation exists in some populations, whereas no correlation is observed in others.

Prediction of T1D as the Basis for Disease Prevention

There are different approaches for the identification of individuals at risk for T1D. These approaches are based on family history of T1D, genetic disease markers, autoimmune markers, or metabolic markers of T1D. These alternatives may also be combined in various ways to improve the predictive characteristics of the screening strategy. The importance of understanding the natural history of immune-mediated prediabetes lies in the development of prevention strategies. Several randomized clinical intervention trials have been concluded and the next generation of such trials will rely upon improved and simplified identification of individuals who are at high risk of progression to T1D. This is essential to ensure that trials have sufficient statistical power to detect a given effect of the intervention within the time available for the study. Such understanding is also needed to avoid exposing those who will not develop T1D to the risk of adverse effects of the intervention. In addition there is accumulating evidence that, at the onset of T1D, preservation of even low levels of insulin secretion has multiple benefits in terms of improved glycemic control and prevention of complications.[29]

Prevention of T1D: Current Status

Although the process by which pancreatic beta cells are destroyed is not well understood, several risk factors and immune-related markers are known to accurately identify first-degree relatives of patients with T1D who may develop the disease. Since we now have the ability to predict the development of T1D, investigators have begun to explore the use of intervention therapy to halt or even prevent beta cell destruction in such individuals. The autoimmune pathogenesis of T1D determines the efforts to prevent it. Susceptible individuals are identified by searching for evidence of autoimmune activity directed against beta cells. While direct evaluation of T-cell activity might be preferable, antibody determinations are generally used for screening because these assays are more robust. Antibody titers are often used in combination with an assessment of the genetic susceptibility, primarily evaluated by HLA typing.

Interventions are generally designed to delay or prevent T1D by impacting some phases of the immune pathogenesis of the disease. As discussed below, current trials are attempting to modify the course of disease progress at many points along the presumed pathogenic pathway. Most prevention trials include only relatives of T1D patients, a group in which risk prediction strategies are most established. Trials in genetically at-risk infants evaluate whether avoiding one of the putative environmental triggers for T1D can delay or prevent its onset.

Primary Prevention

Primary prevention identifies and attempts to protect individuals at risk from developing T1D. It can therefore reduce both the need for diabetes care and the need to treat diabetes-related complications.

T1D is relatively easy to prevent in animal models of the disease, including an array of therapies is effective. However, the mechanism of prevention is usually poorly defined, and there is a lack of surrogate assays of the immune response to define which therapies are likely to prevent diabetes in humans. Inability to define surrogate assays probably results from a fine balance of the immune system, so that even with inbred strains of animals, only a subset progresses to diabetes, and thus, relatively small changes in immune function may prevent disease. These observations have led to the hypothesis that identifying children at a very high genetic risk for diabetes, prior to development of measurable beta cell autoimmunity, and treating them at that point may be a more effective means of diabetes prevention. Studies for the primary prevention of T1D, i.e., prior to the expression of islet autoantibodies, are currently being designed and implemented. These studies target young children at a very high genetic risk for T1D and propose treatments that are very safe. These studies require large-scale screening to identify high-risk subjects and a follow-up over a long period of time to observe the outcome of anti-islet autoimmunity as a surrogate marker for the disease and onset of hyperglycemia as final end point.

A large worldwide trial called *TRIGR* and a small one in Italy called *PREVEFIN* aim to answer the question of whether cow's milk administered in early life is diabetogenic and whether the use of cow's milk hydrolysate can protect from the disease (Figs. 49.1 and 49.2). The rationale behind the use of cow's milk hydrolysate for primary prevention of T1D is based on several epidemiological and in vitro studies indicating that intact cow's milk, if given before 3 months of age, may induce an immune response toward beta cells.[30]

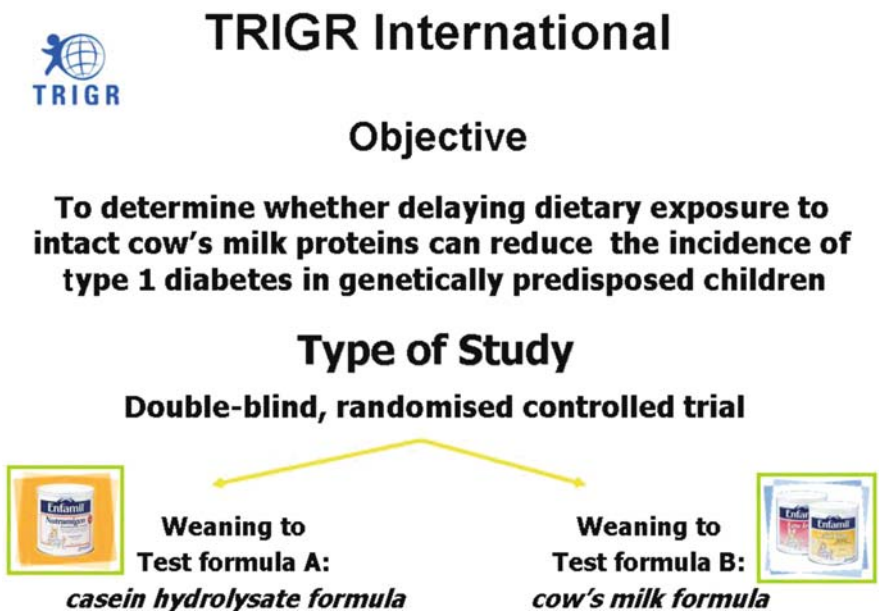

Fig. 49.1 Design of the trial to reduce the incidence of type 1 diabetes in the genetically at-risk study (TRIGR study)

TRIGR is a randomized double-blind intervention study with the intention to treat as well as statistically analyze the incidence of predictive islet cell autoantibodies vs. the actual occurrence of clinical diabetes in two treatment groups (Fig. 49.1).[31] This trial, which investigates cow's milk as an environmental factor, has several key features. First, it is designed to intervene specifically in first-degree relatives of T1D patients. The newborns enrolled must have a genotype with diabetogenic HLA alleles without protective alleles and a mother, father, or a sibling who suffers from T1D. Second, the sample size is highly significant since previous trials were considered to estimate the number of newborns necessary to participate. This is an international trial, and recruitment has been carried out during a 2-year period in nine European countries, six major centers in the USA, 12 centers in Canada, and three centers in Australia. Due to statistical considerations, the frequency of the high-risk HLA genotype, consent, and dropout rates, the trial required initial access to 8000 pregnancies which ultimately yielded 5156 infants necessary for randomization. Each formula milk used in the two treatment groups is a nutritionally complete infant formula. The study formula contains extensively hydrolysed casein as the protein source, vegetable oils as fat source, glucose polymers, and modified starch as carbohydrate source.

The control formula is a mixture of a standard commercial cow's milk-based formula powder made by the same company plus casein hydrolysate powder in a 4:1 ratio designed to mask the flavor and smell distinctions between the two study formulas. The major outcome for the first phase is the frequency of T1D-associated islet cell autoantibodies and/or the development of clinical diabetes by the age of 6 years. The outcome of the second phase will be the manifestation of T1D by the age of 10 years. The manifest diabetes outcome will be assessed as the proportion of subjects in each group who develop T1D, as well as age at diagnosis.

In *PREVEFIN*, the first national preventive trial of T1D in Italy, newborns from the general population (over 10,000 screened at birth) screened for the presence of the high-risk genotype HLA-DR/DQ for T1D (DRB1*-DQB1*0201/DRB1*04-DQB1*0302) (Fig. 49.2).[32] This high-risk genotype has been found to have a frequency of only 0.9% in the general Italian population, lower than in other Caucasian populations, thus explaining the

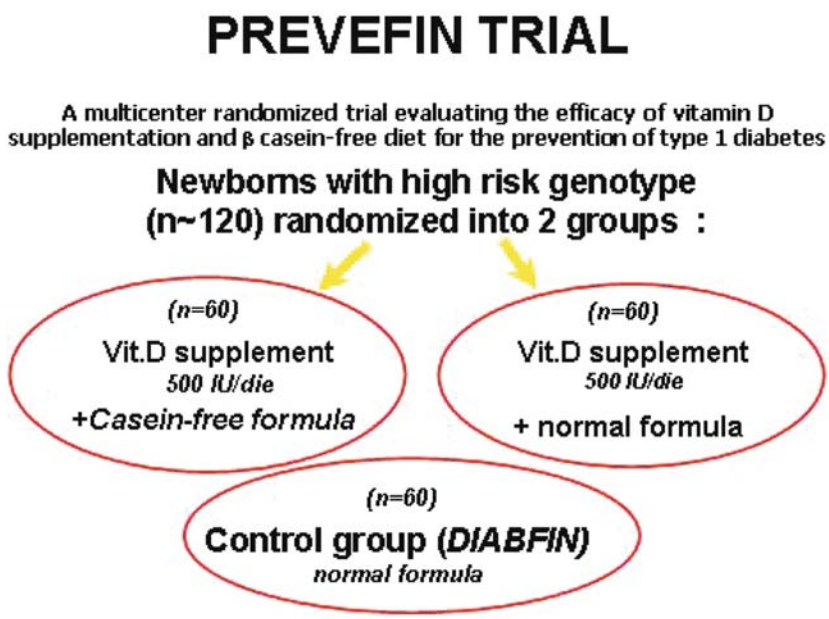

Fig. 49.2 Design of the PREVEFIN project: Prevention of type 1 diabetes in the general population

low incidence of T1D in continental Italy. Many centers are participating in the project which will yield information concerning acceptability of and compliance with early childhood intervention to prevent T1D. The HLA screening is performed within the first 2 weeks of life, so that randomization occurs before 1 month of age. High-risk newborns are recruited into two treatment arms from the time mothers have stopped breast-feeding or if they do not breast-feed. Treatment consists of (i) normal cow's milk formula with vitamin D supplementation (500 IU/day) or (ii) cow's milk hydrolysate with vitamin D supplementation (500 IU/day) continued for up to 1 year. Vitamin D supplementation was included following recent evidence in animal models and humans that administration of this vitamin to newborns can reduce T1D incidence later in life. Detection of islet cell autoantibody and later insurgence of diabetes will be used as an end point. Subjects who participate in similar project, called *DIABFIN*,[33] form a control groups, where newborns with the same high-risk HLA genotype as in the *PREVEFIN* trial are being followed for the appearance of islet cell autoantibodies and diabetes. While the proposed trial may not allow all questions to be fully answered, this national collaborative network will provide safety, efficacy, and logistic data necessary to design a phase III trial.

Another pilot study, called *BABYDIET*, is currently underway to determine whether primary intervention through delayed introduction of dietary gluten is feasible and may reduce the incidence of islet autoimmunity in high-risk first-degree relatives of patients with T1D (Fig. 49.3).[34] The study is based on the premise that introduction of foods containing gluten or cereal before the age of 3 months is associated with an increased risk of islet autoimmunity in childhood. Newborn children are eligible if they are younger than 3 months, are offspring or siblings of patients with T1D, and have HLA genotypes that confer a high T1D risk.

Finally, the Diabetes Prediction and Prevention Project (*DIPP Study*)[35] is a longitudinal study on T1D prediction and prevention carried out in the university hospitals of Turku, Tampere, and Oulu (Finland) (Fig. 49.4). The aim of the study is to investigate longitudinally the dietary factors in relation to the development of diabetic autoantibodies and clinical T1D. The diet of the children is followed up by a structured questionnaire and by 3-day dietary records at various ages. A food frequency questionnaire is applied for studying the dietary intake of pregnant and lactating mothers.

The aims of this project are (1) to identify infants at increased genetic risk for T1D from the general population at birth, (2) to monitor such children for the appearance of diabetes-associated autoantibodies, to identify those at high risk to develop clinical disease, and to characterize the natural course of T1D, (3) to identify the environmental factors inducing the seroconversion to autoantibody positivity in children at increased genetic risk; and

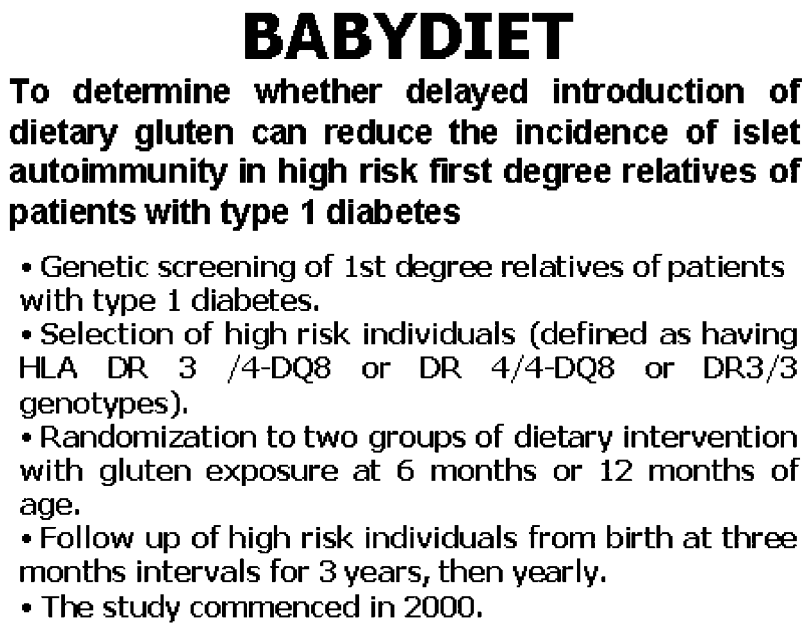

Fig. 49.3 Design of the BABYDIET study

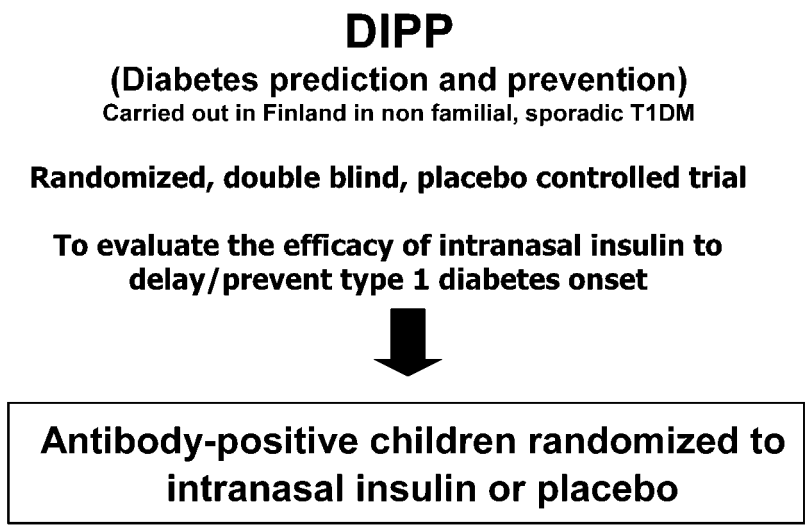

Fig. 49.4 Design of the diabetes prediction and prevention study (DIPP study)

(4) to evaluate whether it is possible to delay or prevent progression to clinical T1D by daily administration of intranasal insulin.

Whereas points 1–4 have been fulfilled and useful information has been obtained, the trial with intranasal insulin did not show any beneficial effect of this treatment in preventing the disease (unpublished data, oral presentation, 9th International Congress of the IDS and ADA Research Symposium, Miami, FL (USA) 14–18 November 2007).

In conclusion, since the failure of *ENDIT* and *DPT1* trials (see section "Secondary Prevention") in preventing the onset of T1D in subjects who are beta cell autoantibody positive, interest has switched to prevention trials starting *before* islet cell autoimmunity has developed. These primary prevention trials of T1D offer an exciting

view of how our knowledge of the pathogenesis of this disease can lead to the possibility of intervening at birth. There is still a long way to go; however, the rationale is sound and the prospects seem good.

Secondary Prevention

Secondary prevention aims to reduce the incidence of T1D by stopping progression of beta cell destruction in individuals with signs of such a process. A number of early studies of secondary prevention were carried out, in some cases interesting results were obtained (as in the case of gluten-free diet study), but the majority of these studies suffered from the limitation of the inadequate dimension of the population in the study or an insufficient follow-up time. To this end consortia of investigators have been created, extended to numerous centers, with the objective to generate the required critical mass for the development of studies with sufficient numbers of subjects at risk for T1D.

European Nicotinamide Diabetes Intervention Trial (ENDIT)

The *ENDIT* study conducted predominantly in Europe examined whether nicotinamide could lead to a reduction in the rate of progression to T1D in at-risk relatives of T1D probands. Over 40,000 first-degree relatives aged 5–40 years were screened in centers in Europe and North America. The study was designed to recruit at least 422 subjects with ICA titers $\geq$20 JDF units to be randomized to either a nicotinamide- or a placebo-treated group. With an expected rate of progression to diabetes of 40% in the placebo arm, the proposed 5-year observation period should have allowed a 90% power to observe a 35% reduction in the incidence of disease.[36–38]

The rationale for using nicotinamide was derived from studies conducted in animal models and humans. In both the streptozotocin- and the alloxan-induced models as well as the NOD mouse and BB rat, nicotinamide was shown to protect the animals from diabetes. In human studies, nicotinamide was reported to preserve C-peptide levels, and, in high-risk ICA-positive subjects, to delay progression to T1D.[39] Similarly, in studies of both at-risk relatives and the general population carried out in New Zealand, nicotinamide appeared to have a protective effect on the subsequent development of T1D.[40]

Several mechanisms have been proposed to explain the protective effect of this antioxidant. One model of beta cell death proposes that, whatever the nature of the beta cell insult is (e.g., cytokine/toxin), nitrous oxide is generated leading to DNA strand breaks, activation of poly(ADP) ribose polymerase (PARP), NAD depletion, and cell death. Part of nicotinamide's protective effect is thought to derive from its ability to prevent NAD depletion during DNA repair by inhibiting PARP. In PARP-depleted knockout mice, those susceptible to diabetes were prevented from developing the disease. Other mechanisms, including inhibition of free radical formation, beta-cell regeneration, protection from macrophage-mediated cytotoxicity, suppression of MHC class II expression on islet cells, and suppression of adhesion molecule-1 expression on islet cells, may also be involved.[41]

Despite all these promises based on a sound rationale, nicotinamide treatment at the doses used did not show any significant effect on the primary outcome – progression to T1D. A total of 159 participants developed the disease within 5 years of randomization to treatment, 82 (30%) in the active treatment group and 77 (28%) in the placebo group. The unadjusted Cox proportional hazard estimate showed no difference between the placebo and the nicotinamide groups on an intention to treat basis. Nor any difference was found between groups after adjustment for age at baseline, glucose concentrations at 2-h glucose in the OGTT, and number of islet autoantibodies. The proportion of relatives who developed diabetes within 5 years was almost identical in those treated with nicotinamide and those treated with placebo, and there was no suggestion of a treatment effect in any of the subgroups defined by well-established markers of additional risk.

A useful message of this trial has been that large-scale collaborations were essential to move things forward and that the place for single-center trials was limited.

DPT-1 Trials

The Diabetes Prevention Trial – Type 1 (*DPT-1*) consisted of two clinical trials that sought to delay or prevent T1D. Nine medical centers and more than 350 clinics in the USA and Canada took part in the two trials of the *DPT-1*.[42–44]

Individuals who were eligible for testing were identified as follows: age 3–45 years, with a brother or sister, child or parent with T1D, and age 3–20 years, with a cousin, uncle or aunt, nephew or niece, grandparent, or half sibling with T1D. Those who met these criteria had ICA antibodies measured. To be eligible, a subject had to be positive for ICAs.

Animal research and small studies indicated that small, regular doses of insulin could prevent or delay T1D in subjects at risk. One *DPT-1* trial tested whether low-dose insulin injections could prevent or delay the development of T1D in people at high risk for developing T1D within 5 years. The study was divided into three parts: screening, staging, and intervention. Subjects were recruited from study clinics and through media campaigns.

Screening

First-degree relatives, 3–45 years of age, and second-degree relatives, 3–20 years of age, of patients with T1D were screened for islet cell antibodies. Those with an islet cell antibody titer of 10 JDF units or higher were offered staging evaluations.

Staging

Staging confirmed the presence of islet cell antibodies, measured insulin antibodies, assessed the first-phase insulin response to intravenous glucose, assessed oral glucose tolerance, and determined the presence or absence of HLA-DQA1*0102,DQB1*0602, a protective haplotype, which, if present, excluded subjects from further participation.[43]

Islet cell antibody-positive subjects were then defined as having a high risk of diabetes (a 5-year risk of more than 50%) and were deemed eligible for the parenteral insulin trial if they had a first-phase insulin response below the threshold (as defined below) on two occasions, if their oral glucose tolerance results were not completely normal, or both. Relatives who tested positive for islet cell antibodies and insulin antibodies and who had a first-phase insulin response above the threshold and normal glucose tolerance were defined as having intermediate risk (a 5-year risk of 26–50%) and were deemed eligible for the ongoing oral insulin trial.

Intervention

Subjects identified as having a high risk of T1D were eligible for random assignment to the experimental intervention (parenteral insulin therapy) or to a control group that underwent close observation. Subjects were stratified according to glucose tolerance status (normal vs. impaired or indeterminate) before randomization. Randomization was performed by a central, automated system, was stratified according to baseline glucose tolerance and clinical center, and used blocks of random, variable sizes.

By the time randomization was completed, samples for screening for islet cell antibodies had been obtained from 89,827 relatives. Of these, 84,594 samples were eligible for further study. The remaining samples were excluded because they came from subjects without an identified relative with diabetes or persons whose age was outside the range defined by the protocol. By the end of the enrollment period, 84,228 samples had been analyzed for islet cell antibodies, and 3152 of the subjects (3.7%) were found to be islet cell antibody positive. Of these, 354 (11.2%) were excluded before randomization because they had a fasting plasma glucose level of 126 mg/dl or higher or a glucose level of 200 mg/dl or higher 2 h after oral glucose challenge. These values, if confirmed, are diagnostic of diabetes.

A total of 2103 subjects (66.7% of those who were islet cell antibody positive) underwent staging. On initial intravenous glucose tolerance testing, 535 subjects had a low first-phase insulin response. As staging continued, a total of 372 subjects were classified as having a high risk and were deemed eligible for randomization; of these, 339 underwent randomization (91.1%), 169 were assigned to the intervention, and 170 to observation. There were no statistically significant differences between the treatment groups.

The results demonstrated that insulin, in small doses, can indeed be administered safely to persons who are at risk for T1D. The increase in presumed and definite hypoglycemia among the subjects in the intervention group did not adversely affect cognitive function.

In high-risk relatives of patients with diabetes, the insulin regimen did not delay or prevent the development of T1D.[42] Long-term follow-up to detect any effects on the course of diabetes has begun. There are several potential explanations for the lack of effect observed so far. One is that the intervention took place too late in the disease process to slow down the progression of disease. Studies conducted earlier in the disease process, such as the ongoing DPT-1 oral insulin trial in relatives of patients with T1D who have a projected 5-year risk of 26–50%, may be more successful. Moreover, oral insulin may have a greater immunologic effect, although it does not provide for beta cell rest. In fact, the low-dose insulin used in the trial may have failed to achieve such an effect on beta cells, but the dose was limited by the risk of hypoglycemia. With a different dosing scheme or a different regimen, insulin or insulin-like peptides might alter the course of development of diabetes.

The other study was an oral insulin trial that sought to prevent T1D in subjects with a moderate risk for developing diabetes. The study was divided into three parts: screening, staging, and intervention. Participants were recruited through media campaigns.

Screening

First-degree (ages 3–45 years) and second-degree (ages 3–20 years) relatives of patients with T1D were screened for ICAs. Those with ICA titer $\geq$10 JDF units were invited to undergo staging evaluations.

Staging

Staging confirmed ICA positivity, measured insulin autoantibody (IAA) status, assessed first-phase insulin response (FPIR) to intravenous glucose, assessed oral glucose tolerance (OGT), and determined the presence or absence of HLADQA1* 0102/DQB1*0602 (a protective haplotype that excluded subjects from participation). Relatives who were ICA positive and IAA positive and had normal glucose tolerance were projected to have a 5-year risk of 26–50% (“intermediate risk”) and were eligible for the oral insulin trial. The original protocol had an entry criterion of confirmed (on two occasions) IAA level >5 SD above the mean of the normal reference range (i.e., $\geq$80 nU/ml). This criterion was agreed upon after reviewing data from natural history studies suggesting that a sufficient cut off was >3 SD above the mean of the reference range (i.e., IAA $\geq$39 nU/ml).[43]

Intervention

The study was a double-masked, placebo-controlled, randomized clinical trial, in which participants were assigned to receive capsules of either oral insulin, 7.5 mg of recombinant human insulin crystals (Eli Lilly, Indianapolis, IN), or matched placebo. Subjects consumed the capsule (insulin or placebo) as a single daily dose before breakfast each day, either by taking the capsule or, if the subject could not swallow capsules, by sprinkling its contents in juice or on food. Randomization used a central automated system, stratified by clinical center, using random variable block sizes.

By the end of enrollment, 97,273 samples were analyzed for ICA and 3483 (3.58%) relatives were ICA positive. Of these, 458 (13.1% of ICA-positive individuals) were excluded before randomization because they already had diabetes. A total of 2523 (72.4% of ICA-positive individuals) underwent staging. There were 1844 relatives with intravenous glucose tolerance FPIR above threshold. As staging continued, a total of 388 relatives were classified as intermediate risk and eligible for randomization; of these, 372 were randomized (97% of eligible subjects), 186 to each study arm; there were no statistically significant differences between treatment groups.

In the primary analysis of relatives selected and randomized in DPT-1, oral insulin did not delay or prevent development of diabetes. There was greater variability in the IAA assay for values 39–79 nU/ml than for values ≥80 nU/ml, particularly in confirmation of a positive result (98.7% overall confirmation for values ≥80 nU/ml compared with 70.6% for values 39–79 nU/ml). This prompted comparison of the rate of evolution of diabetes by entry IAA level. The cohort with confirmed IAA ≥80 nU/ml (the original entry IAA criterion) progressed to diabetes at a faster rate than those subjects who did not have confirmed IAA ≥80 nU/ml. In addition, those with confirmed IAA ≥ 80 nU/ml had other risk characteristics that suggested more rapid evolution to diabetes, including younger age, greater likelihood of having other antibodies, and greater loss of beta cell function (lower levels of plasma C-peptide in response to several provocative challenges).[44]

The effect of intervention in each of these two subgroups was further evaluated.

The group with confirmed IAA ≥ 80 nU/ml showed a beneficial effect of oral insulin, whereas the group who did not have confirmed IAA ≥ 80 nU/ml showed a trend suggesting a detrimental effect of oral insulin.[44] This group also had a much lower overall rate of development of diabetes. Thus, the significance of this finding is unclear but is reminiscent of the adjuvant-induced acceleration of diabetes observed in the BB rat.[45]

In conclusion, neither low-dose insulin injections in subjects at high risk for developing T1D nor insulin capsules taken orally by those at moderate risk for T1D were successful at preventing or delaying the disease.

The two large trials of secondary prevention of T1D (nicotinamide and insulin) did not modify progression to T1D. These trials did however show that large-scale international collaborative prevention trials are possible and that current methods for predicting T1D are accurate. They also prepared the way for a worldwide network to allow intervention trials to be completed as rapidly and efficiently as possible. Continuing on from the basis that it is possible to do high-quality studies to test agents that might potentially prevent diabetes, the National Institutes of Health (NIH) in the USA set up a network of centers across North America and other parts of the world to coordinate a program of prevention studies (*TrialNet*).[46]

Tertiary Prevention

Tertiary prevention is aimed at delaying or preventing the development of complications in subjects who already have T1D. A landmark trial investigating patients with T1D showed that good glycemic control can reduce the likelihood of microvascular complications leading to blindness or kidney disease, but the trend toward a decrease in macrovascular disease was not statistically significant. Diabetes education of health-care professionals and those affected by diabetes plays a key role in the tertiary prevention of the disease. Tertiary prevention is identified by the maintenance of the residual beta cell function present at disease onset and can be realized by immune suppression or immune modulation since the time of clinical diagnosis of T1D.

The best results in this field were obtained 20 years ago with the use of *cyclosporine* [47–49] subsequently abandoned because of transient benefits and undesired adverse effects.

In the following years none of the several treatments that have been proposed has obtained appreciable results except for *nicotinamide*.[50,51]

Clinical trials have demonstrated that, after a year of continuous treatment with nicotinamide, baseline C-peptide levels are slightly higher compared with subjects who did not take this substance.[52] Only recently, experience obtained with the use of the *anti-CD3 monoclonal antibody* in two studies (one in the USA and the other in Europe) has revitalized the interest in this type of interventions.[53,54] The first one was an early-phase clinical trial that tested anti-CD3 in patients with newly diagnosed T1D. The drug, a modified form of anti-CD3 antibody that minimizes first-dose side effects, was studied by comparing 12 subjects aged 7–30 years who were treated with the antibody to an equal number of patients in a control group who did not receive the drug. One year

after treatment with anti-CD3, the treated patients produced more insulin and needed less insulin therapy than the untreated patients. Retention of even some insulin production is an important clinical goal in the treatment of patients with T1D, since, in general, most patients with the disease eventually lose the ability to make insulin entirely and need to rely completely on injected insulin to maintain metabolic control. Those who received the antibody treatment also had better HbA1c levels. The anti-CD3 was designed to act on the immune system's T cells in a more specific manner than previous attempts at immune intervention in early diabetes. However, adverse effects of anti-CD3 are quite significant and include fever, rash, anemia, nausea, vomiting, and joint pain. The study's encouraging results have led to new trials involving additional patients.

Recently, there has been growing interest in *vitamin D* and its active metabolites in relation to T1D and its immune pathogenesis. Vitamin D metabolites have been shown to exert several immunomodulatory effects, and 1,25-dihydroxyvitamin D3 [1,25-(OH)2D3] can either prevent or suppress autoimmune encephalomyelitis, inflammatory bowel disease, and T1D.[55]

Recent data in humans demonstrated that reduction in vitamin D supplementation is associated with a higher risk of the disease, whereas its supplementation is associated with a decreased frequency of T1D.[56]

Based on this rationale, an open-label randomized trial was designed to determine whether supplementation with the active form of vitamin D (*calcitriol*) at diagnosis of T1D could improve parameters of glycemic control.[57,58] The secretion of C-peptide as an index of residual pancreatic beta-cell function was the primary end point, with HbA1c and insulin requirement as secondary end points. The aim of this study was to investigate whether supplementation with the active form of vitamin D (calcitriol) in subjects with recent-onset T1D protects residual pancreatic beta-cell function and improves glycemic control (HbA1c and insulin requirement). In this open-label randomized trial, 70 subjects with recent-onset T1D, mean age 13.6 $\pm$ 7.6 years, were randomized to calcitriol (0.25 μg on alternate days) or nicotinamide (25 mg/kg daily) and were followed up for 1 year. Intensive insulin therapy was implemented with three daily injections of regular insulin + NPH insulin at bedtime. No significant differences were observed between calcitriol and nicotinamide groups with respect to baseline/stimulated C-peptide or HbA1c 1 year after diagnosis, but the insulin dose at 3 and 6 months was significantly reduced in the calcitriol group. In conclusion, at the dosage used, calcitriol had a modest effect on residual pancreatic beta-cell function and only temporarily reduced the insulin dose.[57]

Another important aspect is related to 25 and 1,25 (OH)2D3 plasma levels in subjects with recent-onset T1D, as low levels of these two compounds have been detected[59] and may influence the effect of calcitriol therapy. Whereas the administration of the former may restore low levels of 25 (OH2)D3, the latter is the compound of choice for beta cell protection because of its immune modulatory action. Based on these data another clinical trial was designed and it is now ongoing. It is a double-blind study in which calcitriol is compared with placebo in recent-onset T1D subjects (less than 6 months from diagnosis) in whom residual C-peptide secretion is still detectable and above 0.3 nM. Primary end point of this trial is to estimate the effectiveness of calcitriol in the protection of beta cell function. Secondary end point is to find out if the administration of calcitriol could determine an improvement of metabolic control with consequent reduction of the insulin dose.

The TrialNet Network (Fig. 49.5)

T1D *TrialNet* is a group of studies which aim to examine the development, prevention, and early treatment of T1D. The goal of TrialNet is to perform intervention studies to preserve insulin-producing cells in individuals at risk for T1D and in those with new-onset T1D and to identify individuals "at risk" for developing this disease. Risk is based on positive islet cell autoantibodies or other autoimmune markers and results of oral and intravenous glucose tolerance tests.

Specific study interventions are determined by TrialNet investigators. Each protocol is thoroughly reviewed by an Institutional Review Board (IRB) before approval is given to start recruitment to make sure that the participant is fully protected and not exposed to unnecessary risks. TrialNet is jointly funded by The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), The National Institute of Allergy and Infectious Diseases (NIAID), The National Institute of Child Health and Human Development (NICHD), The National Center for Research Resources at the NIH [which provides support through its General Clinical Research Centers (GCRC)

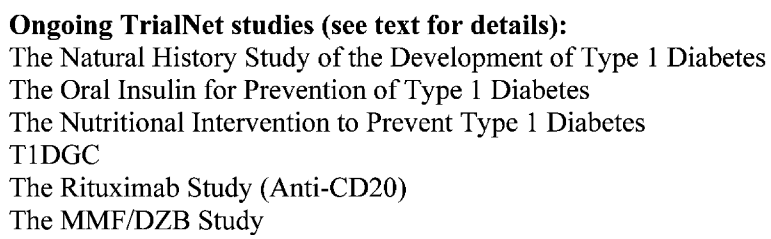

Fig. 49.5 Ongoing TrialNet studies

Program], Juvenile Diabetes Research Foundation International (JDRF), and American Diabetes Association (ADA).

TrialNet conducts multiple clinical trials with investigators from 18 clinical centers in the USA, Canada, Finland, the UK, Italy, Germany, Australia, and New Zealand. Several studies are conducted in patients with newly diagnosed T1D, as well as in relatives of people with T1D who are at greater risk of developing the disease. Two types of studies are envisaged: (a) Natural History Studies which will provide information about risk factors associated with developing T1D and (b) Diabetes Intervention Studies which will test either treatments to delay or prevent the onset of T1D or treatments to preserve remaining insulin secretion in subjects recently diagnosed with T1D.

Ongoing Studies (Fig. 49.5)

(1) *The Natural History Study of the Development of Type 1 Diabetes (currently recruiting)* (http://www2.diabetestrialnet.org/nhx) will study subjects at increased risk for T1D to learn more about how T1D develops. The screening phase of the Natural History Study identifies people at increased risk for developing T1D. Subjects who qualify and choose to participate in the Natural History Study may also be offered an opportunity to enter a diabetes prevention study in the future. To be screened, at least one of the two conditions below must be fulfilled:
   - 1 to 45 years of age and have a brother, sister, child, or parent with T1D
   - 1 to 20 years of age and have a cousin, aunt, uncle, niece, nephew, half sibling, or grandparent with T1D

(2) *The Oral Insulin for Prevention of Type 1 Diabetes Study (currently recruiting)* (http://www2.diabetestrialnet.org/oins) TrialNet has launched a clinical study of oral insulin to prevent or delay T1D in at-risk subjects. The goal is to prevent T1D or to delay it as long as possible. Results from the recently completed study (DPT-1) suggest that oral insulin might delay or prevent T1D in some individuals found to be at high risk. Enrolled subjects will be allocated to one of the two arms of the study – oral insulin or placebo.

(3) *The Nutritional Intervention to Prevent Type 1 Diabetes Study (currently recruiting)* (http://www2.diabetestrialnet.org/nip). The Nutritional Intervention to Prevent Type 1 Diabetes study will help to learn more about a dietary compound, docosahexaenoic acid (DHA), which will be given to pregnant mothers in their third trimester and infants less than 5 months of age. This research is being done as a pilot study, which is a "test run" to find out if it is possible to do a larger study. Pregnant women in their third trimester (more than 24 weeks) may enroll in the study if the baby they are expecting has a relative (mother, father, sister, brother, half sister, or half brother) with T1D. Babies up to 5 months old may also be enrolled in the study if they have a relative with T1D. Pregnant and nursing women will take four capsules a day during the third trimester. Capsules will contain either DHA or a placebo.

(4) *T1DGC (currently recruiting)* (http://www.t1dgc.org/home.cfm). Type 1 Diabetes Genetics Consortium (www.t1dgc.org) is a group of diabetes researchers from around the world who have come together to collect blood samples and information from families with T1D. The T1DGC is a NIH- and EU-funded collaborative effort to develop resources for the scientific community to identify genes influencing a subject's risk

of developing T1D. Network Centers are located in the Asia-Pacific (Melbourne and Australia), European (Copenhagen, Denmark, and Italy), North American (Seattle, WA, USA), and the UK (Cambridge, England) regions. The T1DGC is currently collecting data and blood samples (DNA, plasma, serum and cell lines) on 2800 affected-sibling pair families (two affected siblings, and biological parents and up to two unaffected siblings, if possible). The aims of the study are as follows: (a) to detect the effects of HLA and other candidate regions/genes on the signals from the genome screen, all samples will be genotyped for HLA class II and class I genes (DRB1, DQB1, DPB1, DPA1, A, B, C), INS, and CTLA4 polymorphisms that have previously been implicated in susceptibility to T1D; (b) to refine the localization of the five most promising regions identified from linkage and association studies; and (c) to aid in the confirmation and identification of diabetes susceptibility genes within linked regions, the Consortium will use existing and planned resources of single case families (trios, including an unaffected sibling when available) and sets of T1D cases and nondiabetic controls to carry out detailed disease association analyses.

(5) *The Rituximab Study (anti-CD20) (this study is no longer recruiting patients)* (http://www2.diabetestrialnet.org/anti). This study is attempting to examine if it is possible to stop or slow down the immune system's attack in newly diagnosed T1D patients, so that remaining beta cells can survive and maintain insulin secretion. The goal of the study is to find out if rituximab can prevent further beta cell destruction. Rituximab has been successfully used in other diseases to slow down the immune response. Patients enrolled in the study are randomly assigned to receive rituximab or placebo once a week during the first 4 weeks in the study. Patients eligible for the rituximab study are those diagnosed with T1D within the past 3 months, ages 8–45 years. They need to be islet cell antibody positive and to have evidence of a residual beta cell function.

(6) *The MMF/DZB Study (this study is no longer recruiting patients)* (http://www2.diabetestrialnet.org/mmf). This study will evaluate whether a combination of two drugs can stop the immune system from destroying beta cells in new-onset T1D patients (within 3 months of diagnosis). The two drugs are mycophenolate mofetil (MMF/CellCept®) and daclizumab (DZB/Zenapax®). They both are immunosuppressive agents acting at different levels. The goal is to protect residual beta cell function and improve long-term metabolic control. The study design includes three arms: (a) MMF alone, (b) MMF and DZB together, or (c) placebo.

Patients enrolled in this study are treated for 2 years. MMF or MMF placebo are given as pills (two or three times a day). DZB or DZB placebo are administered intravenously, twice during the first month of the study. In order to be enrolled in this study, patients must have been diagnosed with T1D within the past 3 months and be 12–35 years of age. They need to be islet cell antibody positive and have residual beta cell function.

Summary

The need to obtain consistent results in the difficult field of T1D prevention requires multicentric studies based on international consortia with the aim of being able to achieve of critical mass, statistical power, adequate time of observation, and financing. Examples already mentioned are TRIGR and TrialNet (http://www2.diabetestrialnet.org), a network financed from the NIH and other agencies that has inherited the net formed in the DPT-1 study.

New clinical trials of secondary and tertiary prevention are planned based on the administration of autoantigens (oral or inhaled insulin or GAD) of immune suppression or immune modulation drugs (mofetil mycophenolate, anti-CD25, anti-CD20, anti-CD3 antibodies, and anti-lymphocytes) and of agents potentially able to stimulate a regeneration of the beta cell mass (GLP-1 and analogous). From all these protocols it is reasonable to expect to obtain not only important information on the effectiveness of several treatments but also to learn more about the pathogenesis of the disease. Therefore, prevention of T1D appears at the moment realistically achievable in a near future. Programs of primary prevention must be directed to subjects with a family history for T1D or to those at high risk from the genetic point of view. Moreover, newborns and young adults with

HLA DR3/DR4 who are antibody positive must be carefully followed up with programs of secondary prevention. All the studies must however assure safety and good compliance.

In conclusion, it is necessary to widen the number of clinical trials aimed to develop techniques for prevention of T1D. These studies, however, must include a greater number of subjects and to involve several international centers to achieve the critical mass required for statistically meaningful results.

Acknowledgments We would like to thank Juvenile Diabetes Research Foundation (JDRF), National Institute of Health (NIH) Consortia, Centro Internazionale Studi Diabete (CISD), Diabete e Metabolismo (DEM) Foundation, and University Campus Bio-Medico that support clinical research on T1D in our University Hospital.

Useful Websites

http://www2.diabetestrialnet.org
http://www2.diabetestrialnet.org/nhx
http://www2.diabetestrialnet.org/oins
http://www2.diabetestrialnet.org/nip
http://www.t1dgc.org/home.cfm
http://www2.diabetestrialnet.org/anti
http://www2.diabetestrialnet.org/anti
http://trigr.epi.usf.edu/

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