Type 1 Diabetes Mellitus: Primary, Secondary, and Tertiary Prevention

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ABSTRACT

We have entered the era of clinical trials to prevent type 1 diabetes mellitus (T1DM). Before 1922, when insulin was first given to a patient with diabetes, a diagnosis of T1DM was considered a death sentence. Advances in treatment for subjects with diabetes are not yet sufficient to prevent the deleterious impact of diabetes on both day-to-day activities and the early morbidity and mortality still associated with the disease. We now understand a great deal about blood glucose regulation and potential health complications associated with long-term T1DM, but the mystery of why, or the pathogenesis of this devastating disease, remains elusive. Great strides toward unraveling this mystery have been made over the past several decades. Even without definitive answers, we are moving from the period of discovery and animal research to the era of clinical trials. In this review, we wish to convey the palpable excitement in the field. It is time to determine if we can safely change the course of T1DM.

Key Words: Type 1 diabetes, clinical trial, autoimmunity, prevention.

Although diabetes was known in ancient times, it was not until 1889 that Von Mering and Minkowski at the University of Strasbourg were able to demonstrate that the removal of the pancreas in dogs caused diabetes.1 It then took 33 years before this discovery translated into therapy when Banting and Best identified, isolated, purified, and treated the first subject with insulin. It was not until the 1970s that evidence convincingly demonstrated that insulin-dependent diabetes was an immune-mediated disease. The evidence included measurements of autoantibodies to islet cells2,3 (and subsequently insulin4 and others5–7), pathology reports describing insulitis in pancreatic specimens,8,9 and associations of the disease with genes that encode cell-surface antigen-presenting proteins [the human leukocyte antigen (HLA) system or HLA type].10–12 In the 1980s, reports of islet cell and insulin antibodies together with deficiency of insulin secretion were noted in patients prior to the onset of clinical disease.13 The Eisenbarth model of disease, describing a preclinical period of autoimmune beta cell destruction followed by overt disease, was widely accepted.14,15 During the same era, advances in immunotherapy, reports and then standardization of measurements of additional autoantibodies, and the ability to change the course of disease in the 2 animal models (nonobese diabetic mouse and biobreeding rat) led to the development of clinical trials to do the same. As illustrated in Figure 1, increased understanding of the natural history of the T1DM disease process expanded the concept of prevention to include primary prevention, in which the goal is to stop the initiation of autoimmunity, secondary prevention, in which the goal is to stop the progression of immune-mediated beta cell destruction and prevent clinical disease; and tertiary prevention, with the aim of easing day-to-day management of diabetes and diminishing complications.

NATURAL HISTORY OF DISEASE

The incidence of T1DM in the general Caucasian population is about 0.3%. In contrast, the overall risk among family members is about 15 times greater. Thus, although the majority of individuals who develop T1DM have no family members with
disease, the majority of the data about the natural history of disease come from studies of family members. A large amount of such data has confirmed that a small percentage of individuals at genetic risk develop autoimmunity. This autoimmunity is detected first by the presence of autoantibodies and second by the loss of beta cell function assessed by impaired insulin secretion and subsequently abnormal glucose tolerance. Overt clinical diabetes occurs only after the remaining beta cell function is insufficient to prevent hyperglycemia. At clinical onset, beta cell function is further transiently depressed. For many individuals, a temporary improvement in glucose tolerance is seen within the first few months of diagnosis—the honeymoon period. The honeymoon period is characterized clinically as requiring decreased exogenous insulin and metabolically by improvements in both insulin action and secretion. Whether the honeymoon period is due to the resolution of the acute effects of metabolic decompensation on beta cells and resolution of insulin-sensitive tissues or the immune-mediated cytokine impairment of beta cell function is unknown. Post-diagnosis, immune-mediated beta cell dysfunction and destruction continue, as measured by decreased responses to beta cell insulin secretagogues.

This overall description of the T1DM disease process masks the heterogeneity evident in clinical practice. Some individuals progress to clinical T1DM in infancy; others do so during late adulthood. Whether this is because the disease process is more rapid in younger children or they start with less islet reserve is unknown. Post-clinical diagnosis, about 15% of individuals still have measurable beta cell function even 5 years later, and recent data suggest that small amounts of insulin secretion may persist for decades in selected patients. Overall, however, although the vast majority of patients present with significant amounts of beta cell function at diagnosis, this is gradually and inevitably lost over time.

Although our current knowledge about the natural history of the disease allows for the selection of subjects for clinical trials, further understanding of the causes of the disease will need to take into account emerging information. For example, the incidence of T1DM is increasing worldwide. The increase is particularly evident in the very young. Such an increase suggests environmental triggers during pregnancy or early life. Although such triggers have been previously explored, an ongoing study (The Environmental Determinants of Diabetes in the Young) joins the efforts of international investigators to better understand environmental causes of autoimmunity in newborns. Another relatively recent observation is that there has been an apparent change in the HLA type among individuals being diagnosed with T1DM and that the increase in disease is often in those subjects with moderate-risk or low-risk genotypes. Our developing understanding of the natural history of T1DM must also take into account the influence of lifestyle behaviors and obesity on the disease course. Along with standard assessments of the HLA type, autoantibody status, and metabolic tests, lifestyle data are being collected prospectively as

Fig 1. Stages of Type 1 Diabetes Prevention. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
part of another large international effort, the Type 1 Diabetes TrialNet Natural History Study. Both of these large international studies are also collecting DNA, RNA, and peripheral mononuclear blood cells for use by the research community in exploring the etiopathology of the disease.

PREVENTION STUDIES: RATIONALE AND FEASIBILITY

Studies of the primary, secondary, and tertiary prevention of T1DM are summarized in Table 1.

Primary Prevention

A completely safe, inexpensive, and easily administered therapy to all newborns is theoretically possible. The safety and feasibility bar would need to be quite high because one would be treating 300 infants to prevent 1 case of diabetes. Such a universal approach could be less expensive than a more targeted strategy as it would obviate the need for screening tests. The rationale for such primary prevention is obvious: the elimination of T1DM. However, because there is, as yet, no therapy that has been shown to prevent disease, enrollment in a clinical primary prevention trial currently requires more selective targeting. HLA testing of either the general population (ie, no family members with T1DM) or infants with relatives with the disease can identify those with high-risk or moderate-risk genotypes. Among first degree relatives, about 20% of infants with high risk will develop autoimmunity within the first 2 years of life. Although details of study design are beyond the scope of this review, if one expects the therapy being tested to reduce autoimmunity by a moderately large amount, current knowledge and technology demonstrate that primary prevention studies are feasible. This is clearly illustrated by the ongoing Trial To Reduce Insulin-Dependent Diabetes in the Genetically at Risk (TRIGR), which is testing whether early exposure to cow’s milk influences diabetes risk. Epidemiology data both support and refute an effect of early introduction of cow’s milk as a risk factor for islet autoantibody development. TRIGR is a multinational effort that has screened newborns for genetic risk and enrolled more than 2000 in a placebo-controlled, randomized trial. TRIGR screening and enrollment were completed 1 year early, and the results of this study should be available within the next few years.

Another potential dietary intervention relies on data indicating that the early introduction of cereal and gluten is associated with islet autoimmunity. These observations led to the BabyDiet study, a pilot, primary prevention trial testing whether the development of autoantibodies differs in babies randomized to early (at 6 months of age) or late (12 months of age) introduction of gluten. This study is underway in Germany.

Another pilot study, which began in 2007 and is called the Nutritional Intervention To Prevent Diabetes Study, has been designed to test the hypothesis that omega-3 fatty acids may protect against autoimmune disease. Epidemiological evidence supports this hypothesis, in that a change in Western diets from anti-inflammatory acids to proinflammatory acids over the past century has coincided with an increase in the incidence of T1DM. Also, increased levels of proinflammatory prostaglandin synthase are seen in children with diabetes. Furthermore, a population-based, case-controlled study has suggested that cod liver oil may be protective against the onset of T1DM. In the TrialNet-sponsored Nutritional Intervention to Prevent Diabetes (NIP) primary prevention study, mothers with genetically at-risk fetuses are being given omega-3 fatty acid supplementation in the third trimester of pregnancy. These infants or others found to be at high genetic risk also receive supplements for the first several years of life.

There has been increasing attention paid to the intersection of epidemiologic data suggesting that vitamin D insufficiency is a risk factor for T1DM and basic science studies demonstrating multiple effects of vitamin D on immune and metabolic function. Recent pilot studies have indicated that vitamin D can be safety administered even to the very young, and a primary prevention trial may be considered by TrialNet in the near future.

These efforts all point to worldwide acceptance of primary prevention as the safest way to stop the disease and, moreover, demonstrate that such studies are feasible.

Secondary Prevention

The feasibility of secondary prevention studies was demonstrated during the past decade with the successful enrollment of 3 large, multicenter trials of family members identified as being at risk for progression from autoimmunity to clinical disease. Two of these studies were conducted in North America under the auspices of the Diabetes Prevention Trial–Type 1 Diabetes (DPT-1). In 1 study, islet cell antibody (ICA)–positive relatives with markedly abnormal insulin secretion or abnormal glucose tolerance were enrolled in a randomized
Table 1. Type 1 Diabetes Mellitus Prevention Studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Primary Prevention (Infants and Pregnant Women)</th>
<th>Enrollment Status</th>
<th>Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIP</td>
<td>Docosahexanoic acid capsules versus placebo</td>
<td>Open</td>
<td>DiabetesTrialNet.org 1-800-HALT-DM1</td>
</tr>
<tr>
<td>TRIGR</td>
<td>Bovine protein (cow’s milk) or hydrolyzed casein formula</td>
<td>Closed</td>
<td>trigr.epi.usf.edu</td>
</tr>
<tr>
<td>BabyDiet</td>
<td>Early (6 months) or late (12 months) introduction of gluten</td>
<td>Closed</td>
<td>DiabetesTrialNet.org 1-800-HALT-DM1</td>
</tr>
<tr>
<td>Pre-POINT</td>
<td>Oral and nasal insulin</td>
<td>Not yet opened</td>
<td>JDRF.org</td>
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Secondary Prevention (Relatives of Individuals with T1DM)

<table>
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<th>Enrollment Status</th>
<th>Contact</th>
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</thead>
<tbody>
<tr>
<td>Oral insulin</td>
<td>Oral insulin capsules or placebo</td>
<td>Open</td>
</tr>
<tr>
<td>INIT-II (Australia)</td>
<td>Nasal insulin</td>
<td>Open</td>
</tr>
<tr>
<td>Natural History Study</td>
<td>Determine risk for entry into trials</td>
<td>Open</td>
</tr>
<tr>
<td>Anti-CD3 in very high risk subjects</td>
<td>hOKT3y1 (ala-ala)</td>
<td>Not yet opened</td>
</tr>
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</table>

Tertiary Prevention (Individuals with T1DM)

<table>
<thead>
<tr>
<th>Study</th>
<th>Enrollment Status</th>
<th>Contact</th>
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<tbody>
<tr>
<td>Abatacept</td>
<td>CTLA4-Ig versus placebo</td>
<td>Open: ages 6–45</td>
</tr>
<tr>
<td>ABATE study</td>
<td>hOKT3y1 (ala-ala)</td>
<td>Open: ages 8–30</td>
</tr>
<tr>
<td>Thymoglobulin</td>
<td>ATG versus placebo</td>
<td>Open</td>
</tr>
<tr>
<td>IL-2 and rapamycin</td>
<td>Phase I trial</td>
<td>Open: ages 18–45</td>
</tr>
<tr>
<td>Anti-CD20</td>
<td>Rituximab versus placebo</td>
<td>Closed</td>
</tr>
<tr>
<td>MMF/DZB</td>
<td>Three-arm trial: MMF/DZB, MMF/placebo, and placebo/placebo</td>
<td>Closed</td>
</tr>
<tr>
<td>Anakinra</td>
<td>IL-1B receptor agonist versus placebo</td>
<td>Not yet opened</td>
</tr>
<tr>
<td>Metabolic intervention</td>
<td>Closed-loop glucose/insulin and real-time glucose monitoring versus usual care</td>
<td>Not yet opened</td>
</tr>
<tr>
<td>GAD65 in alun</td>
<td>rhGAD65 (alum) versus placebo</td>
<td>Not yet opened</td>
</tr>
</tbody>
</table>

On-going or planned government or non-profit sponsored primary, secondary, or tertiary prevention trials.

Abbreviations: ABATE, Autoimmunity-Blocking Antibody Therapy for Tolerance in Type 1 Diabetes; ATG, anti-thymocyte globulin; DVDC, Diabetes Vaccine Development Center; DZB, daclizumab; GAD65, glutamic acid decarboxylase 65; IL, interleukin; INIT-II, Intranasal Insulin Trial II; MMF, mycophenolate mofetil; NIP, Nutritional Intervention To Prevent Diabetes; Pre-POINT, Primary Oral/Intranasal Insulin Trial; rhGAD65, recombinant glutamic acid decarboxylase 65; T1DM, type 1 diabetes mellitus; TRIGR, Trial To Reduce Insulin-Dependent Diabetes in the Genetically at Risk.

trial. These high-risk individuals were expected to have a greater than 50% risk of progression to clinical disease within 5 years.42 The other DPT-1 trial enrolled subjects with ICAs and insulin autoantibodies (IAAs) in a double-masked, placebo-controlled trial. These subjects were estimated to have a 25% to 50% 5-year risk of diabetes.43 The European Nicotinamide Diabetes Intervention Trial (ENDIT) was another double-masked, placebo-controlled trial in antibody-positive relatives.44 Although the intervention tested in each of these studies failed to delay or prevent the onset of disease, the trials demonstrated the feasibility of secondary prevention studies. Although large numbers of relatives needed to be tested (>100,000 in the DPT-1 trial), sufficient subjects were enrolled, and study

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compliance was extremely high. Moreover, these large studies confirmed the prediction algorithms. The 5-year risk of diabetes was found to be 60% in the high-risk DPT-1 group and 35% in the intermediate-risk group. Importantly, these studies also confirmed the benefit to subjects participating in such trials, in which they are closely monitored for the development of diabetes. Almost two-thirds of those who developed diabetes were diagnosed during routine follow-up visits when they were asymptomatic, and this prevented the morbidity often associated with diabetes onset.

Because of the large numbers of subjects involved, data from ENDIT and DPT-1 have proved to be valuable for additional insights into the natural history of disease progression. For example, subjects with abnormal glucose tolerance at the time of enrollment [eg, impaired fasting glucose (>110 mg/dL), indeterminate glucose tolerance (30-, 60-, or 90-minute glucose >200 mg/dL), or impaired glucose tolerance (2-hour glucose, 141–199 mg/dL)] were noted to have an extremely high risk of progression (~80% 5-year risk). Furthermore, subjects who do progress to diabetes almost always have normal or impaired fasting glucose when their 2-hour value on the oral glucose tolerance test is >200 mg/dL and they do not have abnormal hemoglobin A1c until their fasting glucose is diagnostic of diabetes. Because this pattern is uncommon in the progression to clinical diabetes among those with T2DM, it may teach us about the relative contribution of impaired secretion and action to glucose tolerance status. Interestingly, although diminished insulin secretion is the sine qua non of autoimmune diabetes, little additional change in beta cell function can be detected in the year preceding diagnosis. This suggests that other factors such as insulin resistance may play a role in subjects reaching the tipping point of diagnostic hyperglycemia. Thus, secondary prevention trials are feasible, they have a compelling rationale in that delaying or preventing onset of clinical disease and close monitoring for progression are of clear benefit to individuals, and these studies provide critical data to exploit as new therapies are developed.

**Tertiary Prevention**

The third area for T1DM prevention is tertiary prevention. As noted previously, at the time of diagnosis, almost all individuals continue to make significant amounts of insulin. Tertiary prevention aims to preserve remaining beta cell function. Because preservation of beta cell function after diagnosis will not cure the disease, it is worthwhile to consider why such studies are important. Data supporting such studies come from a cross-sectional analysis of individuals with and without residual beta cell function as well as data obtained from a retrospective analysis of the Diabetes Control and Complications Trial (DCCT) study. The landmark DCCT trial demonstrated that intensive therapy diminished both microvascular disease (retinopathy and proteinuria) and macrovascular disease (cardiovascular endpoints). However, these benefits were achieved at the risk of severe hypoglycemia, illustrating the Faustian inverse relationship between the risk and benefits of tight diabetes control. In the presence of residual beta cell function, this relationship no longer exists. Thus, a retrospective analysis of DCCT data found that those who had residual beta cell function within the intensively treated group had markedly less hypoglycemia and fewer complications that those without residual secretion. A C-peptide value greater than 0.2 pmol/mL was found to be clinically significant. Other smaller studies have demonstrated the same thing, that residual beta cell function reduces hypoglycemia and complications. Although there remain important questions to answer (is there more benefit for C-peptide levels >0.2 pmol/mL, and how long must C-peptide be preserved to provide the observed benefits?), these data provide a compelling rationale for tertiary prevention studies to determine if therapy could prolong insulin secretion.

In addition to this rationale, it is generally felt that the immune-mediated beta cell destructive process is the same before and after diagnosis. Thus, a therapy that blocks beta cell destruction in tertiary prevention will likely be effective prior to disease onset and thus prevent clinical disease. Because thousands of individuals are diagnosed yearly, the research community can conduct multiple trials in this population, more rapidly determining the risks and benefits of a particular therapy than is possible in either primary or secondary prevention. These trials present their own challenges, including identifying and enrolling subjects soon after a diagnosis fraught with physical and emotional stress. However, because a key element of well-conducted tertiary prevention studies requires intensive diabetes control, all subjects benefit from the close attention and additional resources that come from participation in a clinical trial.

**Autoimmunity and Immune Response**

The role of the immune system is to maintain the delicate balance between effective responses against
harmful pathogens and malignant cells and tolerance to self. Most self-reactive T cells are removed or become quiescent either centrally in the thymus or peripherally as mature T cells. Animal studies have suggested that residual self-reactive T cells can remain dormant because of low avidities of their T cell receptors (TCRs) for self-antigens, lack of costimulation from antigen-presenting cells (APCs), seclusion of self-antigens, or down-regulation of the effector T cells through regulatory T cell activity. Which, if any, of these steps breaks down in human type 1 diabetes is not known. Furthermore, although the self-reactive effector T cells likely cause beta cell death, it is felt that the B cell arm of the immune system must also be engaged to propagate the deleterious response. It is likely that the controls on the immune response have to fail in multiple steps to result in autoimmune disease and that there is no one unique cause of autoimmunity.

In the face of imperfect knowledge of human disease, the choice of intervention generally relies on data from animal models of diabetes and/or data from other human autoimmune diseases. To understand these options, a simplistic concept of the immune response is illustrated in Figure 2. In general, a foreign (or, in the case of autoimmunity, self) protein or antigen is processed by an APC, which then presents the antigen in the context of the major histocompatibility complex to a T cell by interacting with a specific TCR, which includes the CD3 surface protein among others. This initial signal is then followed by secondary interactions between the APC and T cell. These costimulatory molecular pairs include the CD28 and CD40 ligands on the T cell interacting with CD80/86 and CD40 on the APC, respectively. Both are required for the production of an effective immune response; in the absence of costimulation, TCR signaling alone results in anergy or a lack of responsiveness to the signal. Intracellular pathways then respond, eventually propagating the immune attack through the release of cytokines and rapid antigen-specific T cell expansion. As this T cell effector part of the response resolves, a regulatory T cell response ensues, shutting down the immune attack.

From this description, it is apparent that multiple therapeutic targets are possible. One could affect the antigen itself, so that interaction with the immune response would result in anergic or tolerogenic signal. Therapies that alter the first or second signals between the APC and T cell or that have an impact on APC or T cell survival or proliferation could also disrupt the immune attack. Furthermore, manipulation of cytokine responses or an alteration of the balance between T effector and T regulatory cells would be expected to have efficacy in reducing autoimmunity. A full description of the myriad of therapies under development is beyond the scope of this review. Instead, we provide a brief overview of the therapies in recent, ongoing, or soon to begin clinical trials.

**Antigen-Specific Therapies**

**Insulin**

In prospective studies of offspring of mothers with type 1 diabetes, anti-insulin antibodies are usually the first autoantibodies detected, and in animal models and human T cell response to insulin and proinsulin, peptides have been reported. These studies emphasize the importance of these antigens in the disease process and underlie the rationale for insulin-based clinical trials to interrupt beta cell immune destruction. DPT-1 tested the hypothesis that the administration of small doses of subcutaneous insulin in conjunction with a once yearly 4-day infusion of intravenous insulin would alter the natural history of disease in those at high risk of progression (>50% over 5 years). The hypothesis was that this early insulin treatment would act as an antigen to dampen the immune response and/or that it would induce beta cell rest; both had been demonstrated in the animal models to have efficacy in stopping disease. Unfortunately, this parenteral arm of DPT-1 did not prevent or delay the onset of disease, possibly because the dose of insulin used was limited to alleviate potential concerns about hypoglycemia.

A second trial involving insulin was conducted by DPT-1 and included those relatives at intermediate risk of disease (ICA- and IAA-positive relatives with 25%–50% 5-year risk). This study was based on the concept of oral tolerance. The theory of oral
tolerance is based on the understanding that the gut immune system serves a different purpose than the immune system elsewhere. The gut immune system is primarily designed to diminish the immune response. Anyone who has watched a toddler surreptitiously put debris into his mouth can understand this teleologically. If the gut’s immune system responded to everything that it encountered, everyone would be continually allergic. Elegant animal studies have suggested that responses to antigens in the gut involve antigen-specific T cells that release regulatory cytokines. Thus, delivery of insulin to the gut results in a breakdown into peptide fragments, which, while metabolically inactive, are recognized by the immune system. Insulin peptide-specific T cells with regulatory characteristics then travel to the islet, where they dampen the ongoing beta cell attack, thus inducing tolerance.

In the primary analysis of relatives selected and randomized in this DPT trial on the basis of ICA positivity with IAA \( \geq 40 \) nU/mL, there were no untoward side effects from therapy, and unfortunately, oral insulin did not delay or prevent the development of diabetes. Interestingly, in a post hoc analysis, there was evidence of heterogeneity of effect within the study cohort according to the level of IAA. The subgroup with confirmed IAA \( \geq 80 \) nU/mL not only progressed to diabetes at a faster rate than those subjects who did not have confirmed IAA \( \geq 80 \) nU/mL but also showed a potential beneficial effect of oral insulin: a delay of diabetes of more than 4 years! Such a result was clearly not only statistically significant \( (P = 0.015) \) but also of marked clinical significance as one can easily visualize by imagining a teen whose diagnosis is delayed until young adulthood. However, as this was a post hoc analysis, this result can be deemed only to be hypothesis-generating. This is the rationale underlying the new study testing whether oral insulin can delay or prevent diabetes. In this ongoing TrialNet oral insulin study, antibody-positive relatives with the appropriate risk profile are randomly assigned to once daily oral insulin capsules or placebo. Recruitment for this secondary prevention study is ongoing.

A pilot study is under development using oral insulin in a primary prevention trial (PrePoint Oral/Intranasal Insulin Trial). This study will enroll genetically at-risk infants to determine if oral insulin could prevent the development of autoantibodies and thus diabetes onset.

The Finnish Diabetes Prediction and Prevention Study (DIPP) also tested whether antigen-based insulin therapy would result in secondary prevention. In this trial, antibody-positive young children received nasal insulin or placebo, but unfortunately this therapy also did not delay or prevent disease in these very high risk subjects. Study details have not yet been published, but it is likely that the dose used may have been a factor in this trial.

A pilot study using nasal insulin in older antibody-positive relatives was reported by Harrison et al. to show a skewing of the immune response consistent with tolerance induction. A full trial to test whether this will change the course of the disease is underway in Australia and New Zealand (Intranasal Insulin Trial II) (INITT II) and is sponsored by the Diabetes Vaccine Development Center (DVDC).

**Glutamic Acid Decarboxylase**

Although its exact role in the pathogenesis of diabetes is unclear, an important autoantigen against which antibodies are detected in about 70% of patients with type 1 diabetes at the time of diagnosis is the enzyme glutamic acid decarboxylase. Glutamic acid decarboxylase is present in the islets as well as the central nervous system and testes.

Diamyd Corp. has developed a vaccine consisting of a highly purified and unmodified form of recombinant glutamic acid decarboxylase 65 formulated in aluminum hydroxide (alum). As with all antigen-based therapy, the goal is to alter the immune system’s recognition and response to the antigen and induce a state of tolerance to further assault. In this preparation, the alum serves as an adjuvant to induce a protective immune response. In a tertiary prevention study in adults with so-called latent autoimmune diabetes of adults, administration of this subcutaneous vaccine was safe. In unpublished data from a study in youth with T1DM, 2 doses of this vaccine a few months apart appeared to preserve beta cell function in comparison with those treated with placebo. The effect was particularly pronounced in those entering the study soon after diagnosis. TrialNet will soon be starting a clinical trial using 3 doses of subcutaneous recombinant glutamic acid decarboxylase 65 in alum in a double-masked tertiary prevention trial. Individuals within 3 months of diagnosis will be eligible to participate.

DVDC is also sponsoring a phase I trial testing the safety of administration of unique peptides from additional islet autoantigens. They were selected after elegant studies identifying these antigens as being naturally processed and presented by the body. These dose escalation safety studies are ongoing in the United Kingdom (http://www.dvdc.org.au/index.php).
**Immunosuppressant/Immunomodulatory Therapies**

**Thymoglobulin**
Thymoglobulin is a Food and Drug Administration (FDA)–approved global immunosuppressant drug. Possible mechanisms of action include T cell clearance from the circulation and suppression of T cell activation, homing, and cytotoxic activities. It therefore blocks the T cell/APC activation at multiple points. Clinically used as induction therapy in organ transplantation, short-term treatment is designed to rapidly decrease autoreactive T cells. The Immune Tolerance Network (ITN) is sponsoring a phase II tertiary prevention trial in recently diagnosed individuals to determine if such aggressive therapy given over only a short period of time will effectively decrease the immune assault on the beta cells without unexpected side effects. This trial is ongoing and includes subjects within 6 weeks from the onset of their diabetes.

**Mycophenolate Mofetil**
Mycophenolate mofetil (MMF) is an FDA-approved immunosuppressant drug in clinical use to control autoimmune disease (eg, rheumatic disease) and to prevent rejection in organ transplantation. This oral medication inhibits the de novo pathway of guanosine nucleotide synthesis. Although other cell types can use a salvage pathway, T and B cells rely on the de novo pathway. Thus, MMF blocks T and B cell proliferation. Mild side effects such as gastrointestinal upset and diarrhea may be seen upon initiation of treatment, but prolonged therapy is well tolerated. TrialNet has recently finished recruitment for a 3-arm, placebo-controlled tertiary prevention trial using MMF as well as daclizumab (DZB). The concept is that short-term treatment (2 years of therapy) with MMF would sufficiently quiet the destructive immune response and allow the normal balance between effector and regulatory cells to be restored.

**Daclizumab**
DZB is a monoclonal antibody that inhibits interleukin-2 (IL-2)–mediated activation of lymphocytes. The IL-2 receptor consists of 3 components, \( \alpha \), \( \beta \), and \( \gamma \) chains, and it appears on the cell surface of activated T cells. Upon engagement with IL-2, binding increases T cell growth and proliferation. DZB binds to the \( \alpha \) chain of the IL-2 receptor, thus blocking this response. DZB is an intravenous drug FDA-approved for the prophylaxis and treatment of kidney transplant rejection. For the TrialNet tertiary prevention study, DZB/placebo was administered on 2 occasions 2 weeks apart. Well tolerated, this drug is expected to augment the effectiveness of MMF alone. Results of this multicenter TrialNet (TN) study are expected in 2009.

A phase I trial in adults with diabetes who still have residual beta cell function is testing the safety of the combination of IL-2 and rapamycin therapy under the auspices of the ITN. Although both IL-2 and rapamycin are FDA-approved drugs, they have never been coadministered. As noted previously, IL-2 serves to increase the growth and proliferation of all lymphocytes. This includes effector and regulatory cells. New knowledge about differences in the intracellular signaling pathways between effector and regulatory cells led to the observation that the immunosuppressant drug rapamycin would inhibit growth only of the effector cells.\(^{55,66}\) Thus, the rationale behind the combination therapy is that IL-2 will drive both effector and regulatory cells, whereas rapamycin will keep effector cells in check, effectively increasing activated regulatory cells and thus inducing a tolerant state. Rapamycin is an orally administered, FDA-approved immunosuppressant drug widely used in organ transplantation. For this study, it is given for 3 months. IL-2 is administered as a subcutaneous injection 3 times a week for a month. Impressive results in animal models with this combination\(^7\) support this phase I trial. Results should be available in 2009.

**Anti-CD3 Monoclonal Antibodies**
The key role of CD3 in the TCR complex was recognized decades ago and led to the development and use of an antibody designed to target and then destroy activated T cells with CD3 on the cell surface. Although effective in organ transplantation, marked side effects limited its use. Two new antibodies to the CD3 molecule were then developed, both designed to bind CD3 on activated T cells and modulate the response without causing massive T cell death, which was thought to underlie the side effects seen with earlier versions. Importantly, animal studies indicated that these new anti-CD3 drugs seemed to have a prolonged effect after short administration, thus inducing a tolerant state.\(^{68}\) Several trials involving the treatment of subjects with new-onset T1DM have been conducted with a modified anti-CD3 monoclonal antibody. In an initial ITN-sponsored phase I trial, hOKT3y1 (ala-ala) (now being developed as Teplizumab by Macrogenics) was administered over a 14-day course to recently diagnosed individuals between the ages of 7 and

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in a randomized but not placebo-controlled trial. After 1 year of follow-up, C-peptide levels from the 4-hour mixed meal tolerance test were improved in 71% of treated subjects (n = 21) versus 21% of the control subjects (n = 19). Although there remained a difference between the groups with respect to C-peptide levels after 2 years, analysis of the follow-up data revealed a delayed but parallel decline in C-peptide response in comparison with controls.69,70 This observation underlined the rationale for an ongoing follow-up ITN-sponsored trial (Autoimmunity-Blocking Antibody Therapy for Tolerance in Type 1 Diabetes) (ABATE), in which individuals will receive 2 courses of anti-CD3 treatment a year apart to assess whether the effect on beta cell function can be prolonged. Subjects 8 to 30 years old and within 6 weeks of diagnosis are eligible to enroll. The second modified anti-CD3 molecule, ChAglyCD3 (called TRX4 and under clinical development by Tolerx Co.), was studied as tertiary prevention in adults recently diagnosed with T1DM. Beta cell function in the treated groups was greater than that in controls, and there was a decrease in insulin requirement without differences in diabetes control as measured by hemoglobin A1c.71 Side effects of both new anti-CD3 monoclonal antibodies, such as mild aches, fevers, and rashes, are likely attributable to cytokine release and are managed with anti-inflammatory and antihistamine therapy. These drugs are still in the early stages of drug development. Neither of these new antibodies is FDA-approved, although both continue in clinical trials.

The results from the tertiary prevention trials have led to the development of a TrialNet study in secondary prevention. In this study, antibody-positive relatives at very high risk of developing diabetes (ie, those with confirmed abnormal glucose tolerance who have about an 80% risk of disease) will be randomly assigned to treatment or not to determine if therapy can delay or prevent disease. Adults will receive placebo infusions, whereas children ages 8 to 18 will be randomized to treatment or close observation. Because the very high risk relatives are routinely being identified through the ongoing TrialNet natural history study, it is anticipated that enrollment will begin in 2008 and last about 2 years.

**CTLA4-Ig**

CTLA4-Ig (Abatacept) is an FDA-approved drug clinically used in autoimmune disease, including rheumatoid arthritis, and being tested in inflammatory bowel disease, multiple sclerosis, and lupus. This drug blocks costimulation between CD28 on the activated T cell and the APC, thus limiting the immune response. Clinical trials have documented the effectiveness and safety profile in very young children treated for juvenile rheumatoid arthritis. The drug has been administered as a monthly infusion lasting about 30 minutes, and there have been limited side effects associated with the infusion. A double-masked, placebo-controlled tertiary prevention trial of CTLA4-Ig in individuals ages 6 to 45 has just been launched by TrialNet. This study includes subjects within 3 months from their date of diabetes diagnosis with the aim of preserving residual beta cell function.

**Rituximab**

Because convincing evidence in animal models points to the role of T cells in destroying islet beta cells and others in autoimmune disease, it was somewhat surprising to observe that rituximab, a monoclonal antibody that destroys the immune system’s B cells, was effective in patients with autoimmune disease. Originally developed and FDA-approved for the treatment of B cell lymphoma, it is now FDA-approved for the treatment of rheumatoid arthritis. This therapy is being tested in a double-masked, placebo-controlled tertiary prevention trial in recently diagnosed T1DM patients under the auspices of TrialNet. All subjects have been recruited for this trial, and preliminary information on whether this therapy preserves beta cell function is expected by the end of 2008.

**Anakinra**

Anakinra blocks the biological activity of IL-1 by competitively inhibiting IL-1 binding to the IL-1 type I receptor, which is expressed in a wide variety of tissues and organs. Depending on the concentration, this cytokine is known to be deleterious to cultured islet cells.72 It was recently tested in individuals with T2DM. After 13 weeks of therapy, patients had improved beta cell function and improved glycemic control.73 Anakinra will be used in a double-masked tertiary prevention trial sponsored by the Juvenile Diabetes Research Foundation (JDRF) in recently diagnosed subjects in Europe in 2008.

**Other Approaches**

**Metabolic Control**

The aforementioned studies are all primarily focused on interfering with the immune system. However, other factors clearly play a role in the development and clinical course. For example, the notion that residual beta cells would better be able to fight off immune attack if they were rested was
one of the hypotheses that underlined the DPT-1 high-risk prevention trial discussed previously. Considerable data emphasize that hyperglycemia itself is deleterious to beta cells, either directly or indirectly. For example, in the DCCT, subjects in the intensive insulin therapy group maintained better beta cell function.\textsuperscript{74} This has led to the planning of a new tertiary prevention trial using cutting-edge technology to maintain strict glucose control. Subjects within a week of diagnosis will be randomly assigned to usual care or to a closed-loop glucose-measuring, insulin-delivery system as inpatients to be followed by real-time continuous glucose monitoring to optimize insulin delivery. This TrialNet-sponsored study of intensive metabolic control should begin in 2008.

**Beta Cell Growth**

Obviously an intervention that diminishes or blocks the immune assault combined with a therapy that may augment islet beta cell growth and development would be of great interest. Despite a large number of animal and in vitro studies suggesting that glucagon-like peptide 1 mimetics such as exenatide have salutary effects on inhibiting beta cell apoptosis or increasing beta cell mass,\textsuperscript{75} it is not clear whether these effects occur in humans. Furthermore, although animal studies also suggest that epidermal growth factor and gastrin may promote beta cell mass,\textsuperscript{76,77} other data suggest that such therapy would not likely be beneficial in humans.\textsuperscript{78} Nonetheless, it is attractive to test the effects of these clinically available therapies. As such, TrialNet is considering combination trials with anti-CD3 and exenatide and/or antigen therapy in both secondary and tertiary prevention.

**COORDINATED EFFORTS TOWARD THE PREVENTION OF TYPE 1 DIABETES**

**Multicenter Networks**

Clinical trials to stop immune-mediated beta cell destruction are not new (see the reviews in refs. 79–82). Indeed, the cyclosporine trials demonstrated that immunotherapy can have an impact on the disease process.\textsuperscript{83–85} However, most investigators feel that this is indeed a new era. The explosion of knowledge about fundamental aspects of the immune response, the plethora of therapies available to be tested, our increased sophistication about clinical trial design, and the increasing willingness of the diabetes community to participate in such studies underline the enthusiasm for such studies by funding agencies and pharmaceutical companies.

Although important studies are being conducted by individual researchers and groups around the world, the National Institutes of Health is funding 2 clinical trial networks to push the field forward. One, the ITN, is designed to conduct studies across autoimmunity, transplantation, and allergy with the aim of inducing tolerance. On a practical level, this implies using short-term therapy for long-term benefit. T1DM is one of the diseases in the ITN portfolio, and this network is currently conducting several phase I/phase II trials. More information about clinical trials underway through ITN can be found at www.immunetolerance.org.

Diabetes TrialNet is a network of clinical centers working in cooperation with affiliated sites throughout the United States, Canada, Finland, the United Kingdom, Italy, Germany, Australia, and New Zealand. This network, sponsored by the National Institutes of Health, JDRF, and American Diabetes Association, is designed to conduct multiple clinical trials in primary, secondary, and tertiary prevention. TrialNet also conducts other studies with the aim of facilitating standardization of clinical trial endpoints and testing of new assays that may one day serve as surrogate markers of the disease process or treatment effects. Updated information about TrialNet-sponsored research studies can be found at www.diabetestrialnet.org.

The JDRF, in conjunction with the Australian government, also funds another network, the DVDC. The DVDC conducts clinical trials to interrupt beta cell destruction. Information on the DVDC can be found at www.dvdc.org.au.

There are several significant advantages of multicenter networks. First, there can be consistencies in study design that allow for informal comparisons between trials. Second, the networks make primary and secondary prevention studies feasible and tertiary studies more rapidly accomplished. Third, publicly funded multicenter trials undergo a rigorous review before they begin and are carefully regulated. For example, a proposal submitted to TrialNet is reviewed for scientific rigor, ethical justification, clinical feasibility, and prioritization before it moves forward for a steering committee vote. An external advisory committee and independent data safety review board then evaluate the protocols, which are all operated under FDA regulations. No study moves forward without ethical or institutional review board approvals at each participating site. Safety and safety monitoring are important components of all of these studies. This is accomplished through

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an evaluation of clinical and laboratory data by the TrialNet medical monitor and a TrialNet safety committee. For studies involving immunotherapy, infectious disease consultants are readily available as needed, and regular reviews of all data are conducted by an independent data safety monitoring board. Similar structures are in place for other multicenter studies as well. Multicenter studies also provide widespread opportunity for participation by families.

**Role of the Diabetes Clinical Team**

Clinical investigators in T1DM need the help of the diabetes clinical team to let families and individuals with T1DM know that there are opportunities for participation in primary, secondary, and tertiary prevention trials. It is well recognized and understood that many families will decide not to participate in clinical research. However, too many families still are not informed about studies. Many later express frustration that they are outside the window of time from diagnosis and thus may no longer have a choice about participating or that they find out about screening programs for family members only after their second child is diagnosed with diabetes.

During this era of multiple clinical prevention trials, it is too much to ask busy clinicians to keep up with the myriad of approaches being tested. Indeed, this review does not even include all such studies! Rather, the clinician should understand the rationale underlying primary, secondary, and tertiary prevention studies and take an active role in letting patients and their families know about the studies. It may also be necessary to remind families with diabetes that the knowledge gained from these prevention trials may be applied in the future when blocking of autoimmunity is needed for any biological beta cell replacement therapy. The clinician should refer families to the web, let them know about toll-free phone numbers, and encourage them to ask questions.

With your help, we hope to soon close the era of clinical trials and bring about an entirely new treatment paradigm for individuals at risk for or with diabetes.

**DISCLOSURES**

*Potential conflict of interest:* Dr. Greenbaum is Vice-Chair of Diabetes TrialNet, a member of the scientific review for the Immune Tolerance Network, a member of the board of directors of the Diabetes Vaccine Development Center, and is an investigator for clinical trials sponsored by these NIH or non-profit groups.

**REFERENCES**


17. Hoogwerf BJ, Rich SS, Barbosa JJ. Meal-stimulated C-peptide and insulin antibodies in type 1 diabetic


