



GOAL II:

PREVENT OR REVERSE TYPE 1 DIABETES

Recent Scientific Advances

Tolerance and Regulation of the Immune System

Identification of Autoantigens and Improved Tools for the Study of Type 1 Diabetes Onset

Advances Toward Preventing or Reversing Type 1 Diabetes

Research Objectives and Strategies To Achieve Goals

Risk Assessment

- ▶ Identify and Optimize the Detection of Immunologic, Genetic, and Metabolic Markers of Type 1 Diabetes

Immunopathogenesis

- ▶ Understand the Interplay Between Early Environmental Encounters and Immunoregulatory Defects That Results in Beta Cell Destruction in Human Type 1 Diabetes
- ▶ Advance Basic Understanding of Facets of the Human Immune Response (e.g., Regulatory T Cells, Innate Immunity) That Have Recently Been Appreciated as Key Mediators of Beta Cell Destruction

Clinical Trials

- ▶ Identify an Intervention Capable of Long-term Reversal of Recent Onset Type 1 Diabetes Without Concomitant Short- or Long-term Adverse Effects
- ▶ Develop a Safe and Universal Means for the Primary Prevention of Type 1 Diabetes

STRATEGIES TO PREVENT OR REVERSE TYPE 1 DIABETES

INTRODUCTION AND BACKGROUND

The immune system is normally well regulated against the formation of self-directed or “autoimmune” processes due to the body’s remarkable ability to form “tolerance,” a process whereby cells of the immune system are either eliminated or turned off if they react to one’s own cells or proteins. Yet, for unknown reasons, this process of immunological tolerance fails to work properly in persons who develop type 1 diabetes, thereby permitting the self-destruction of beta cells. As discussed in Goal I, research suggests that this autoimmune attack may be triggered and/or exacerbated by as yet unknown environmental factors in people who are genetically at increased risk for developing the disease, but the specific roles of genetics and environment in the pathogenesis of type 1 diabetes remain unclear.

An individual’s level of genetically encoded risk for developing type 1 diabetes aside, the earliest marker that portends ultimate beta cell destruction is the appearance in the bloodstream of antibodies (i.e., autoantibodies) that recognize “self” beta cell proteins. In type 1 diabetes, autoantibodies are not themselves thought to be causative of disease, as they are in myasthenia gravis, for example. Instead, they are thought to result indirectly from the cell-mediated immune destruction of the pancreas, often referred to as the white blood cell response. This is not to say that autoantibodies are without clinical or diagnostic value in type 1 diabetes. Indeed, they have been used as highly effective biomarkers for identifying individuals who are in pre-clinical stages of the disease, and have served in the biochemical definition of the self-proteins that are targets of immunological attack. While many forms of white blood cells play important roles in the autoimmune processes that damage beta cells (e.g., macrophages, dendritic cells), a key role has been suggested for T cells (also called T lymphocytes)—a cell type that, in addition to having destructive capacity, has the potential to limit immune responses.

Based on the present state of knowledge, a cure for type 1 diabetes will hinge on the ability to interrupt the destructive assault by the cell-mediated immune system. Such interruption will be necessary whether the goal is: (1) to stop the disease before it progresses to full-scale loss of pancreatic endocrine function (i.e., avoiding symptomatic onset and need for insulin therapy); (2) to reverse type 1 diabetes; or (3) to prevent the recurring immune attack on islet beta cells following their transplantation into patients with long-standing type 1 diabetes. Indeed, a central problem that must be solved is the development of a method that promotes the induction of immunological tolerance to pancreatic beta cells in people genetically predisposed to type 1 diabetes. It should also be emphasized that basic as well as applied research will be of critical importance for achieving this goal.

Type 1 diabetes research is fortunate to have not just one, but several spontaneous rodent models of the disease, which mimic many aspects of the human disease. These animals serve as excellent surrogates in which to evaluate the mechanisms underlying type 1 diabetes, and can be used for testing agents capable of reversing the autoimmune processes mediating beta cell destruction. Yet, they also have limitations, including incomplete fidelity to human disease. The BB rat and the non-obese diabetic (NOD) mouse are the most prevalent models, at least as evidenced by the number of publications emanating from their use. Both animal models share many characteristics with human type 1 diabetes, including genetic susceptibility by molecules regulating the immune response; white blood cell infiltration of the pancreatic islet cells; disease that is influenced by environmental exposures; and the production of autoantibodies against beta cell proteins. Furthermore, in both models, beta cell destruction can be attenuated through application of agents capable of influencing the immune response. However, several therapies that have been shown to be effective in animal models are not effective in people.

While attempts have been made to turn advances in understanding the autoimmune basis for type 1 diabetes into a cure for the disease for nearly three decades, more progress has been achieved in the last 5 years than in the previous 25 years combined. Only recently have researchers realized that the autoimmune processes associated with type 1 diabetes begin for many in the first months to years of life (i.e., when the aforementioned autoantibodies form). Similarly recent is an improved appreciation of the ability of environmental factors (e.g., diet, viruses) to influence, in a positive or negative way, the rate of progression to type 1 diabetes. Through studies of both humans and of animal models of type 1 diabetes (e.g., BB rats, NOD mice), dramatic improvements have recently occurred in understanding the “basic” immunologic mechanisms that, acting in concert, contribute to the dysregulated immune response that results in loss of tolerance, beta cell destruction, and, eventually, type 1 diabetes.

Tolerance and Regulation of the Immune System:

Recent studies of animal models have provided insights into type 1 diabetes, such as:

- ▶ Ascertaining the physiological locations of the defects that underlie the failure to develop tolerance to beta cells (i.e., the role of the thymus versus cells of the immune system that circulate through the peripheral immune system);
- ▶ Identifying immune system cells that are key to inducing tolerance in type 1 diabetes (e.g., B lymphocytes, dendritic cells, regulatory T cells); and
- ▶ Pinpointing the contributions that various cytokines (i.e., chemical signals of the immune response) make to the onset and progression of this disease.

It is important to note that many of these disease aspects can only be addressed through studies of animal models due to issues of both practicality and technical ability, providing but one of many examples of the importance of animals to type 1 diabetes research. Progress has also occurred toward understanding tolerance and regulation of the immune response in human type 1 diabetes, implicating defects in many cell types (e.g., regulatory T cells, dendritic cells, natural killer T [NKT] cells) as potentially causative in autoimmune disorders such as type 1 diabetes. Similarly, several genes (e.g., *AIRE*, and others derived from the genomic analyses described in Goal I) have been identified that contribute to autoimmune disorders because of their ability to modify immune reactivity.

Identification of Autoantigens and Improved Tools for the Study of Type 1 Diabetes Onset:

For years, researchers have struggled to determine which beta cell proteins are key targets of autoimmune attack. A variety of investigations, in both animal models and humans with type 1 diabetes, now support the notion that the insulin molecule itself is an important, potentially disease-initiating autoantigen in this disease. Additionally, other studies have recently identified islet-specific glucose-6-phosphatase catalytic subunit related protein (IGRP) and dystrophin myotonic kinase (DMK) as antigenic targets of the cellular immune response in NOD mice. There is also continuing interest in the potential role that proteins of neuroendocrine origin may play in the disease (e.g., glutamic acid decarboxylase, IA-2, phogren) in both human type 1 diabetes and in animal models. To a large extent, many of these recent discoveries regarding autoantigen identification were dependent on the development of improved tools for characterizing the immune response associated with beta cell destruction (e.g., T cell tetramer and ELISPOT assays, genetically modified mouse models of type 1 diabetes), as well as on access to human tissues made available for research purposes (e.g., islet cells, pancreas, pancreatic lymph nodes from type 1 diabetes patients). In addition to immune markers, a variety of metabolic markers and their associated tests have proven valuable to studies of human type 1 diabetes. Particularly notable are the recent improvements in the ability of researchers to determine the metabolic activity of individuals with or at-risk for type 1 diabetes (e.g., C-peptide standardization).

Advances Toward Preventing or Reversing Type 1 Diabetes:

Recent years have brought much excitement about possible treatment strategies stemming from proof-of-principle experiments in animal models. These include: anti-CD3, which depletes and/or modifies the function of T cells; CTLA4-Ig, which antagonizes immune activation (e.g., “co-stimulatory blockade”); and anti-thymocyte globulin, which also depletes T cells. In addition, research on immunosuppression associated with the islet transplantation efforts, as described in Goal III, contributes leads for agents that could be used to control autoimmunity in the disease prevention or reversal setting. Those agents that demonstrate adequate safety profiles have and will continue to move forward in human type 1 diabetes clinical trials through such programs as NIH’s Type 1 Diabetes TrialNet or the Immune Tolerance

Network. Anti-CD3 is one example of an agent that has seen experimental translation from animal models to investigations in humans. Two research trials of anti-CD3 reported the ability of this agent to preserve metabolic function when administered to people with recent onset type 1 diabetes. With time, it is hoped that this or other agents will become proven components of a cure for type 1 diabetes by promoting disease reversal.

Studies of animal models of the disease, as well as investigations of its natural history in humans, have generated a number of agents or practices that could be useful for preventing the disease in those with a high likelihood of developing it (e.g., omega-3 fatty acids, cow's milk avoidance, oral or nasal insulin). In some situations, methods used for disease prevention may be similar to or the same as those for type 1 diabetes reversal. However, it also appears that a “one size fits all” approach to type 1 diabetes therapy will not be practical. Studies of animal models suggest that optimizing therapeutic efficacy may depend on tailoring the therapy for each point in the disease process and/or targeting different pathways by combination therapy.

In terms of attempts to prevent the disease, a degree of disappointment obviously surrounds the results of the Diabetes Prevention Trial-Type 1 (DPT-1). This trial was conducted in relatives of type 1 diabetes patients who did not themselves have the disease, but who had signs of autoimmunity. It found that insulin administered via daily injection did not prevent type 1 diabetes in people at increased risk for the disease. However, a number of positive research outcomes were and continue to be seen from that effort. First, the trial instilled an appreciation that very meaningful scientific information can be gleaned from trials, even when prevention of disease may not occur. For example, there was an observable nationwide confirmation of the practical ability to use autoantibody and genetic markers of type 1 diabetes to predict future cases of the disease. Because physicians can effectively identify individuals at increased risk for the disease, they are in a better position to fight the disease when superior interventions are developed. In addition, although injected insulin was ineffective, the trial suggested that oral insulin administration may have a potential benefit with respect to delaying disease in a select group of people identified as being at intermediate risk. This approach will be tested in a future effort using the TrialNet consortium.

RESEARCH OBJECTIVES AND STRATEGIES TO ACHIEVE GOALS

Finding a means to prevent or cure type 1 diabetes will require an accurate assessment of what is truly known about the disease in humans, as well as an organized plan to fill the knowledge voids that stand between the diabetes community and that goal. To that end, the following objectives are critical.

Risk Assessment

DPT-1 affirmed, at a national level, the ability to identify individuals at increased risk for future development of type 1 diabetes. This study—in a patient population of relatives (non-diabetic but having signs of autoimmunity) of type 1 diabetes patients—was built on years of experience in smaller trials indicating the value of screening for type 1 diabetes using combinations of autoantibody, genetic, and metabolic markers for the disease. Despite this success, the prediction of type 1 diabetes largely remains limited both in scope of application (i.e., who is screened) and in the locations in which such testing occurs (i.e., within academic research settings). Furthermore, practical improvements in the technology of disease prediction would be of immense benefit, as would better integration of additional physiological parameters (e.g., body mass index [BMI], age) to enhance existing predictive models.

Indeed, a large majority of studies to date have focused on screening relatives of individuals with type 1 diabetes. This focus is understandable in terms of efficiency (the risk of type 1 diabetes in close relatives of those with the disease is approximately 1 in 25, while in the general population it is around 1 in 300 [16]). However, more than 80 percent of new onset type 1 diabetes patients do not have a known family history of the disease (16). Also, it remains to be seen whether the disease characteristics of patients from the general population differ from those identified in family groups—differences that could have impacts on the efficacy of a proposed treatment or prevention. Hence, it would be wise to initiate studies testing the feasibility of population-based screening in order to identify at-risk individuals from the pediatric population as a whole. Also, while type 1 diabetes screening is efficacious, for the most part it remains a research-based effort performed in a limited number of academic research centers. While such institutions certainly play a key role in type 1 diabetes care, only a small percentage of people receive health care in such facilities. Therefore, it would be valuable to develop point-of-service screening for type 1 diabetes risk, such that these assays could be performed in pediatricians' offices (e.g., using a finger stick blood test). It must be

emphasized that this forward-thinking objective remains research-based and must, if implemented, be undertaken with training and education for health care providers, as well as the general public. Such efforts must also be accompanied by the full intent to investigate issues of patient privacy and the ethical use of this information. Furthermore, the psychological impact of at-risk status and the most appropriate manner for communicating risk must be considered.

Technological improvements must go hand-in-hand with broad access to samples to promote the development and validation of risk assessment technologies. Past experiences suggest that assembling and tracking large collections of samples require considerable investment and buy-in from study investigators from the very beginning, as well as careful consideration of issues related to patients' informed consent and privacy. Large multicenter clinical studies and trials (e.g., TrialNet, TEDDY) are already collecting and archiving sample banks to be made available to the research community. An important priority is to continue and expand these efforts to promote access and efficient distribution of samples to the research community.

Research Objective—Identify and Optimize the Detection of Immunologic, Genetic, and Metabolic Markers of Type 1 Diabetes:

- ▶ *Achieve accurate identification of those at risk in the general population by improved measurement of autoantibodies and other autoimmune markers.*

Intensive efforts should be directed at miniaturizing existing technologies for assessment of immune activities related to development of type 1 diabetes. Specifically, diagnostic tests should be developed that require smaller blood volumes than are currently necessary and permit collection under conditions that do not require vein puncture (e.g., capillary tube collection, spotting of blood samples on filter paper). Such improvements would facilitate more frequent monitoring of patients, leading to discoveries of changes in the immune response that are not currently observed with existing collection schedules (e.g., quarterly, semiannually). Improvements in technologies of a different sort could also enable much needed improvements in assays for anti-insulin autoantibodies, as well as the identification of any additional, previously unknown beta cell autoantigens. As previously indicated, while autoantibodies represent important and proven markers of type 1 diabetes, the processes underlying the disease likely reside in components of the cellular immune response. In a majority of situations, earlier attempts to use cellular immune markers for type 1 diabetes screening have proven diffi-

cult in terms of technical reproducibility and practical issues. This situation must change. Fortunately, new technologies are being developed, which could provide the more powerful biomarkers that are needed. These technologies include genomics (examples provided in Goals I and VI), proteomics (discussed in Goal VI), RNA markers, and the quantitative measurement of cytokines in blood.

- ▶ *Achieve accurate type 1 diabetes risk assessments by exploiting additional genetic markers.*

Given that new technologies will also continue to revolutionize genetics, future studies should determine whether additional genetic markers could refine and improve existing algorithms for type 1 diabetes prediction. Many of the opportunities and challenges within this area of research were described under Goal I. Genetic risk assessment for type 1 diabetes should also be expanded to define the risk for a series of other autoimmune disorders (e.g., celiac disease, Addison's disease, rheumatoid arthritis) that often occur in patients with type 1 diabetes. Such expansion could have the potential benefit of affording primary, secondary, or early tertiary intervention for these related disorders to reduce their disease-associated morbidity and mortality.

- ▶ *Achieve accurate type 1 diabetes risk assessment using metabolic parameters.*

In addition to improvements in immunologic and genetic markers, similar efforts for discovery should be aimed at enhanced understanding of metabolism in the type 1 diabetic setting, in the period prior to symptomatic onset, as well as at disease diagnosis. Specifically, studies should examine a variety of physiologic variables (e.g., age, BMI, insulin resistance), with the aims of improving understanding of their contribution to the heterogeneity of this disease and designing targeted therapies that might prove more effective given a specific set of immunologic and physiologic criteria. Additional efforts should also be directed at continuing the process of standardizing C-peptide response to metabolic stimulation as a measure of beta cell function and addressing outstanding questions, such as: What should be measured? Which test should be used to measure it? When should the test be administered?

Immunopathogenesis

While studies on the natural history of type 1 diabetes have not yet resulted in a means to prevent or cure the disease, they have led to a remarkable improvement in understanding the events prior to the symptomatic onset of disease. As previously mentioned, people identified to be at either low or high

risk in DPT-1 were characterized as extensively as possible (within the logistical, ethical, and scientific constraints), to identify molecular and cellular markers that indicated a high likelihood of progression to overt type 1 diabetes. While the ability exists to stratify individuals at birth for their risk for type 1 diabetes, studies of the natural history of type 1 diabetes in early childhood and adolescence (such as the TEDDY study and the Natural History study within TrialNet) clearly need to continue. Such studies address the need to know more about the role of the environment in the pathogenesis of the disease, as well as to provide a more detailed characterization of the immune system abnormalities that result in beta cell destruction.

Research Objective—Understand the Interplay Between Early Environmental Encounters and Immunoregulatory Defects That Results in Beta Cell Destruction in Human Type 1 Diabetes:

- ▶ *Improve understanding of the interplay between the environment and the immune system, which leads to the autoimmune destruction of beta cells in humans.*

For decades, investigators have sought to identify the “type 1 diabetes virus.” However, as discussed in Goal I, recent research suggests that there is a complex liaison between viral infections and other potential environmental triggers of type 1 diabetes. Only recently have researchers begun to appreciate the possibility, based on animal models, that some environmental agents may not enhance disease progression, but rather, may offer protection from disease. Hence, it has now become of paramount importance to define experimentally the scenarios that can potentiate acceleration of beta cell destruction versus those that can dampen autoimmune beta cell destruction. Because these studies are exceedingly difficult to perform, close collaboration among a number of large, prospective research efforts is necessary, such as the coordination provided by the TEDDY study (described in Goal I). These collaborative efforts will promote efficient investigation of important issues, such as the influence of diet, infection, and psychological stress on the development of anti-islet autoimmunity. It would be beneficial to capture individuals undergoing anti-beta cell autoimmunity at the height of an inflammatory event in epidemiological studies and not only at set-time intervals. Additional reasons to continue studies on the natural history of type 1 diabetes include the need to establish whether type 1 diabetes is, like other immune-mediated diseases, a disease of flares and remissions. Individuals with evidence of autoimmunity progress to diabetes at varying rates. It is currently unknown whether environmental or behavioral factors (e.g., diet, exercise,

psychological stress) influence the progression of the disease. Research needs to address the impact of environmental factors as a trigger for autoimmunity in genetically at-risk individuals (as is being done in TEDDY), as well as the role of environmental and behavioral factors in the progression of the disease in people who have already developed diabetes-related autoimmunity. Finally, researchers need to gain a better understanding of the interaction of the innate and adaptive immune system in disease development, as well as the role of gut immunity in the development of type 1 diabetes.

- ▶ *Create a database of the genes expressed in the pancreas at sequential stages of type 1 diabetes development, as well as accessible tissues involved in the (auto)immune response.*

Substantial research into gene expression and proteomics will be required to translate findings from T1DGC and TEDDY into new molecular diagnostic tests to help physicians predict type 1 diabetes, determine the stage of islet autoimmunity, select preventive measures, and monitor therapies. Some of the genetic markers will be considered as potential therapeutic targets for new drugs. Microarray experiments are providing unprecedented quantities of genome-wide data on gene expression patterns, but the management and analysis of the millions of data points that result from these experiments will require sophisticated new computational tools. These tools should be utilized in studies to: (1) assess levels and patterns of gene expression in each tissue before and after appearance of islet autoantibodies and autoreactive T cells, and before and after candidate environmental exposures; (2) correlate the level and patterns of expression at the mRNA and/or protein level with the genetic and metabolic phenotypes of humans and animal models before and after disease onset; and (3) generate expression analyses from a panel of humans and laboratory animals at different stages of type 1 diabetes. The latter effort should focus on the genes most likely involved in environmental triggering of islet autoimmunity and progression to overt diabetes, to determine the range of sequence and expression variation in these genes and the proteins they encode.

Research Objective—Advance Basic Understanding of Facets of the Human Immune Response (e.g., Regulatory T Cells, Innate Immunity) That Have Recently Been Appreciated as Key Mediators of Beta Cell Destruction:

- ▶ *Improve the understanding of the generation and function of regulatory T cells in type 1 diabetes.*

In the mid-1970s and early 1980s, studies of animal models of type 1 diabetes suggested a key role for T cells in the processes of beta cell destruction. For nearly two decades, the mechanisms by which these cells could act in both a destructive and a protective fashion remained enigmatic. Within the last 5 years, research has highlighted the role of a population of T cells commonly referred to as “regulatory T cells,” a form of the white blood cell that may represent one of the master regulators of the immune response. Studies in NOD mice, BB rats, and human type 1 diabetes patients suggest important pathogenic and therapeutic relationships between these regulatory T cells and disease. It is imperative to determine the role of regulatory T cells in the natural history of type 1 diabetes. Lack of understanding about the cellular immune response in general, and T cells in particular, represents one of the most serious gaps in knowledge that must be filled to realize the goal of prevention and reversal of type 1 diabetes.

► *Develop better assays to measure the autoimmune response and to serve as biomarkers of response to therapy.*

One possible approach to this objective would be to develop assays with animal models, using blood taken from a human patient, to detect and quantify T lymphocytes capable of inducing type 1 diabetes. Such assays could also be deployed to monitor responses to immunologic therapies for type 1 diabetes. These assays would provide benefits by both identifying the most efficacious agents and predicting response to therapy. Improved cellular immune assays are also needed to determine the metabolic and immunologic events that occur during transition from pre-symptomatic to overt disease. Likewise, these assays will be important in determining the relationship between genotype and phenotype in humans, particularly with respect to immunologic function. It should be emphasized that the need for improved assays for these purposes is especially required for monitoring cell-mediated immunity in peripheral blood from patients enrolled in clinical trials. Development of assays of immune activation and/or tolerance is a key objective described in Goal III. It is likely that common approaches can and will be used to study both autoimmunity and alloimmunity relevant to transplantation.

► *Detect and measure the autoimmune response, as well as the mass and function of beta cells, at the level of the pancreatic islet.*

While diagnostic or research-oriented sampling can safely be accomplished in certain cases (e.g., rheumatology, kidney transplantation patients), pancreatic biopsy is neither safe nor practical in individuals with or at risk for type 1 diabetes. However, it is critically important to identify the destructive T cells, as well as the molecules that they recognize, that

infiltrate islets and pancreatic lymph nodes of people who have or are developing type 1 diabetes. Recently, an initiative has put in place an international network of centers with the ability to screen deceased individuals for detectable islet autoantibodies and to obtain from those antibody-positive individuals pancreatic and nearby immunologic tissue. This effort may seem like a “needle in the haystack” problem, but such extensive efforts are worthwhile, given the importance of obtaining this essential material resource. Other efforts have been directed at improving the ability to image *in vivo*, noninvasively and safely, but with high resolution, the degree of beta cell mass and the quantity of islet infiltration and inflammation. Aside from further efforts to understand damage inflicted on beta cells by the immune system, additional studies should be directed toward examining the effect of hyperglycemia, independent of immune attack, on beta cell destruction and growth. Noninvasive imaging of islet cell mass and function, as well as inflammation or immune infiltration, is a goal common to diabetes prevention and reversal, and to islet transplantation efforts.

Clinical Trials

Interestingly, a great many interventions have been shown to be capable of preventing type 1 diabetes in rodent models that spontaneously develop the disease. Fewer have been shown capable of reversing type 1 diabetes in animals, and fewer still have been tested for their capacity to prevent or reverse the disease in humans. Selected examples range from those with a dietary/environmental basis (e.g., nicotinamide, delayed introduction of cow’s milk) and immunosuppression/immunoregulation (e.g., cyclosporine, anti-CD3) to those that have an antigen-specific immunomodulatory function (e.g., oral and subcutaneous insulin).

Considerable evidence suggests that administration of a variety of beta cell autoantigens can delay the onset of type 1 diabetes in animal models of the disease. For example, some studies point to insulin as a beta cell autoantigen with potential pathogenic significance. While the DPT-1 study did not support the ability of injected insulin to prevent type 1 diabetes, a number of distinctions exist between the tested therapy and the use of a putative insulin vaccine. Among them would be aspects related to form (e.g., insulin peptides, the use of adjuvants to stimulate immune responses, route of delivery); function (i.e., type of immune response one wishes to elicit); and time of administration (i.e., early in life versus the late administration employed in the DPT-1). To be clear, studies of autoantigen administration should not be limited to insulin. Moreover, the impact of such trials may extend beyond that

of universal/early administration to therapies of recent onset type 1 diabetes.

The current state of knowledge offers several agents with therapeutic potential, but no single agent is clearly most worthy of testing for the prevention of type 1 diabetes. Thus, achieving this objective will involve multiple clinical trials. Such trials should not only test efficacy in terms of type 1 diabetes prevention or reversal, but also assess the ever important safety considerations and impacts on quality of life. Efforts are currently under way (including Type 1 Diabetes TrialNet and the ITN) to implement well organized clinical trials and to establish and maintain an efficient infrastructure for the identification of populations for participation in research. Moreover, the next phase of type 1 diabetes prevention trials will benefit from lessons learned through previous attempts to prevent or reverse the disease.

Knowledge gains stemming from the NIH-funded Diabetes Control and Complications Trial about the health benefits of even low levels of residual beta cell function are furthering efforts to prevent or reverse type 1 diabetes. Extremely beneficial would be the identification of an intervention, or combination of treatments, capable of either inducing complete disease remission or perhaps prolonging the “honeymoon” phase during which new onset patients still have meaningful beta cell function. Such an interventional strategy could not only have a dramatic impact on a patient’s daily life, but could also delay or prevent the development of complications associated with the disease. Indeed, the development of a method for type 1 diabetes reversal would have an immense impact on newly diagnosed patients with type 1 diabetes.

Research Objective—Identify an Intervention Capable of Long-term Reversal of Recent Onset Type 1 Diabetes Without Concomitant Short- or Long-term Adverse Effects:

► *Standardize trial design and outcome measures.*

Information gathered in clinical trials will be most useful if a standardized approach to data collection is taken and adhered to across the participating clinical centers and even across clinical trial and study consortia. This standardization will require cooperation and communication among researchers at every level. Standardization of measures employed in the trials must be undertaken and implemented in an ongoing way. These include measurements of auto-antibodies, including titers and affinities; cellular-based measures of autoimmunity; measures of inflammation; and

metabolic measurements, including C-peptide and hormone production, insulin usage, and glycemic control. Standardized methods should also be developed for assessing side effects, safety, patient and family acceptance, adherence, burden, satisfaction, and quality of life. Consistent data collection on the characteristics of participants who agree (or refuse) trials and remain (or drop out) would enhance future trial design and planning. Other trial design considerations, such as issues of “effect size” and power calculations, will also need to be examined and implemented consistently across trial consortia.

► *Determine whether combination therapies offer improvements in terms of efficacy over monotherapies directed solely at the immune system.*

As already proven in oncology, combination treatment methods may limit adverse side effects while improving efficacy. One particularly promising combination therapy approach to type 1 diabetes would be to test immunomodulating agents along with potential beta cell “growth factors” (e.g., incretin mimetic, growth hormone). Another example would combine a tolerance induction methodology with an immunosuppressive approach to reverse anti-beta cell autoimmunity. Emphasis should also be given to studies that combine immune intervention agents with drugs that send survival signals to islet beta cells, thereby inhibiting programmed cell death, and leading to a preservation of existing beta cell mass and improved beta cell growth. Antigen-specific interventions should also be combined with nonspecific immunosuppressants. The former have the advantage of site-specific and nonsystemic action, while the latter offer an immediate attenuation of anti-beta cell autoimmunity. Such combination therapies would also be clearly relevant to the field of islet transplantation, as described in Goal III.

► *Identify novel therapeutic agents.*

While it is true that many potential therapies for type 1 diabetes reversal exist, there remains a pressing need for additional candidates, including those that could promote a “costimulatory blockade” or an induction of regulatory T cells. For any effort in this area to succeed, it is important to identify groups (e.g., academic, corporate) that are highly proficient and competent in rational drug design and are willing to work with the type 1 diabetes research community to either create novel immune interventions or find new applications for existing drugs. Such efforts will help overcome existing barriers that inhibit large pharmaceutical companies focused on larger markets from committing to high-risk projects such as some of those described in this Strategic Plan.

- ▶ *Assess the safety of all immunomodulating or immunosuppressive therapies tested in type 1 diabetes.*

Recent research on immunosuppression in autoimmune diseases has revealed not only impressive potential benefits, but also great potential risks. Clearly, the risk/benefit equation in a prevention setting is very different from that in a life-saving organ transplant situation. A major research aim is to analyze the effects of immunosuppression on immunization status, viral activation, or reactivation. For example, one of the most feared complications in a chronic disease such as type 1 diabetes is reactivation of viruses that could have long-term oncogenic potential. Indeed, secondary cancers are a key problem with chronic immunosuppression. For type 1 diabetes therapies currently in development, the potential extent of this problem is not known. Hence, every effort should be made to monitor Epstein-Barr virus, herpes simplex virus, and cytomegalovirus reactivation in ongoing immunosuppression trials. Over the long term, studies of safety should also determine the likelihood of other adverse effects, especially those of renal and cardiovascular origin, given their intimate relationship to sites for type 1 diabetes-associated complications. Indeed, an ethical examination of the fine balance between acceptable side effects and efficacy remains a key issue for any new therapy. Aside from issues of safety, additional studies should evaluate whether the preservation of beta cell function in recently diagnosed patients with type 1 diabetes offers short- and long-term clinical benefit with respect to disease-associated complications, particularly those of retinopathy, nephropathy, neuropathy, hypoglycemia, and quality of life. Finally, psychological outcomes associated with participation in these types of studies and interventions should be investigated to understand their full impact on the individual.

- ▶ *Enhance animal models for the study of relevant immune mechanisms and potential interventions.*

Risks associated with testing interventions in human clinical studies, plus recent advances in animal models, provide ample justification for accelerating development of animal models to study human type 1 diabetes-relevant immune processes and potential interventions. For example, newly derived mouse models with greater fidelity to disease (genetically engineered or transplanted with human molecules and tissues) should be given priority testing for their ability to serve as human surrogates for investigation of therapies aimed at attenuating anti-beta cell autoimmunity. Again, such models are common means to the ends outlined not only for Goal II described here, but also for Goal I

(the evaluation of the human genetic and environmental risk factors) and Goal III (the evaluation of methods and mechanisms relevant to islet transplantation).

Research Objective—Develop a Safe and Universal Means for the Primary Prevention of Type 1 Diabetes:

- ▶ *Further investigate the potential utility of autoantigens as “vaccines” for prevention of anti-beta cell autoimmunity.*

It is possible that future research will show that altering or knocking out islet autoantigens will abrogate islet autoimmunity in animal models such as the NOD mouse, as was the case for insulin. If so, this finding would support the notion that type 1 diabetes can depend on more than one autoantigen, as suggested by the existence of multiple autoantibody and T cell specificities in affected NOD mice. Timing could be important here as well. For example, certain “self” targets may be prominent only in some earlier or later stages of disease progression. Such possibilities, currently being intensively researched, are expected to provide information that will be critical for the design of effective autoantigen-based vaccination strategies (e.g., B-chain of insulin to be studied in the ITN). Furthermore, it is possible that innovative therapies, such as vaccines capable of preventing type 1 diabetes, could be developed without the identification of specific environmental targets or beta cell autoantigens for type 1 diabetes. Thus, vaccination against even causally unrelated agents may, through modulation of the immune response, confer protection against type 1 diabetes. Although efforts directed at such approaches have not been fruitful to date, this remains a potentially valuable area for further research.

- ▶ *Determine the importance of exposure to cow’s milk protein in the development of islet autoimmunity and type 1 diabetes via the Trial To Reduce IDDM in the Genetically At Risk (TRIGR).*

The immature intestine allows leakage of undigested dietary proteins that may be antigenic. Although the causes of diabetic autoimmunity in humans remain controversial, studies in diabetes-prone mice and rats show that hypo-antigenic weaning diets are protective. TRIGR seeks to determine whether the risk of type 1 diabetes is different in genetically susceptible infants who are weaned onto a hydrolysate of cow’s milk formula, in which many of the cow’s milk proteins have been broken down, versus standard cow’s milk formula. In addition to answering this important question, the Trial includes a series of mechanistic studies that will be

conducted among children participating in TRIGR. Samples are being repositied and are available for hypothesis-based research. These studies will complement TEDDY in addressing the possible role of enteroviral infections, dietary factors, and gene-environment interactions that may provide the basis for future clinical trials.

- ▶ *Begin the design and implementation of clinical trials aimed at reducing the impact of environmental factors that trigger islet autoimmunity and type 1 diabetes in utero, during early postnatal life, and later in development.*

Many studies (e.g., T1DGC, TEDDY, TrialNet Natural History Study, TRIGR) are accumulating vast amounts of data and samples that can be used to better define genotypes and

phenotypes in patients with type 1 diabetes and their family members. These data will be important for designing and implementing clinical trials for the translation of study findings, for example, through the TrialNet clinical trials infrastructure. Identification of potential triggers through epidemiological studies could directly lead to the design of clinical trials. For example, if confirmed in other ongoing studies, suggestive data about preventing type 1 diabetes by eliminating or modifying exposure to cereals could be the basis of a future clinical trial. Similarly, identification of an infectious trigger or protective agent could generate clinical trials based on vaccination strategies. Successful prevention strategies could ultimately be implemented in the general population.

