GOAL I:

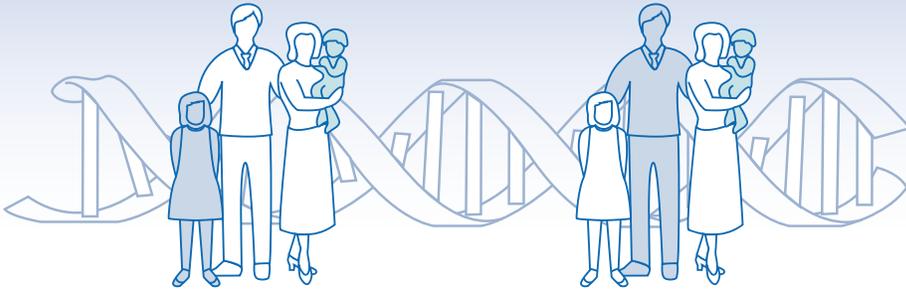## IDENTIFY THE GENETIC AND ENVIRONMENTAL CAUSES OF TYPE 1 DIABETES

Recent Scientific Advances

HLA Genes Contributing to the Risk of Type 1 Diabetes

Contribution of *INS* to Type 1 Diabetes Susceptibility

Other Genetic Factors Associated with Susceptibility to Type 1 Diabetes

Exploration of Human Genomic Regions Associated with Type 1 Diabetes Susceptibility

Genes and Genetic Concepts Discovered in Animal Models of Type 1 Diabetes

Initiation of Studies To Identify Environmental Causes of Type 1 Diabetes

Role of Diet in Type 1 Diabetes

Role of Viruses in Type 1 Diabetes

Role of Stress in Type 1 Diabetes

Research Objectives and Strategies To Achieve Goals

Genetic Causes

- ▶ Create Resources for the Study of Type 1 Diabetes Genetics
- ▶ Identify Human Genes Causing Type 1 Diabetes
- ▶ Use Knowledge About the Genetic Underpinnings of Type 1 Diabetes To Prevent and Treat the Disease

Environmental Causes

- ▶ Monitor Rates of Type 1 Diabetes
- ▶ Assess Environmental Causes of Type 1 Diabetes

STRATEGIES TO IDENTIFY THE GENETIC AND ENVIRONMENTAL CAUSES OF TYPE 1 DIABETES

INTRODUCTION AND BACKGROUND

Type 1 diabetes is characterized by autoimmune destruction of insulin-producing pancreatic beta cells. It has long been known that the likelihood of a person developing type 1 diabetes is higher the more closely related he or she is to a person with the disease. However, even in monozygotic ("identical") twins, this probability is much less than 100 percent (15), and indeed 80 percent of new patients with type 1 diabetes do not have close relatives with the disease (16). The disease also exhibits patterns of outbreaks and seasonality consistent with involvement of infectious agents. These observations suggest that, in addition to a strong genetic component, an environmental factor or factors may also play a role in causing type 1 diabetes.

Type 1 diabetes risk is influenced by multiple genes. Four regions of the genome are known to contain genes related to type 1 diabetes: the major histocompatibility complex (MHC), which includes genes that encode the human leukocyte antigens (HLA) on chromosome 6; the region around the insulin gene (*INS*) on chromosome 11; a region that contains several immune response genes (including *CTLA4*) on chromosome 2;

and the protein tyrosine phosphatase N22 gene (*PTPN22*) on chromosome 1. Studies in both mouse and man indicate there may be as many as 20 other regions containing genes that influence susceptibility to type 1 diabetes. Some of the genes may influence disease only in some populations. In other cases, multiple genes could interact such that the risk associated with specific gene combinations is great, while the risk associated with any one of the genes alone could be small. These factors make identifying the responsible genes challenging.

The environmental contributors to type 1 diabetes are also likely to be complex. A variety of triggers has been suggested. These include viruses, diet, environmental toxins, and stress. However, no definitive proof of a causative link with any of these factors has yet been found. Understanding, preventing, and treating type 1 diabetes critically depends on greater understanding of its causes. The *Special Statutory Funding Program for Type 1 Diabetes Research* has enabled several large-scale clinical studies that will facilitate further understanding of type 1 diabetes genetics, etiology, and prevention.

RECENT SCIENTIFIC ADVANCES

The identification of genes and genetic regions contributing about half the genetic risk for type 1 diabetes has been key to the successful development of clinical trials to test strategies to prevent type 1 diabetes and clinical studies to identify environmental triggers. This genetic information has allowed identification of individuals at risk for type 1 diabetes who might benefit from participation in these clinical studies. Subsequent progress in identifying genes with smaller contributions to risk is opening up new areas of investigation into the pathogenesis of type 1 diabetes and potential new strategies for interventions. The confirmed type 1 diabetes susceptibility genes and gene variants are being employed to describe in detail the genetic and molecular basis for type 1 diabetes pathogenesis in order to identify relevant biological pathways involved as a basis for targeted therapies and drug development.

HLA Genes Contributing to the Risk of Type 1 Diabetes: The genetic basis of type 1 diabetes is complex and likely to be due to genes of both large and small effect and the interaction of these genes. Numerous studies have investigated genetic susceptibility loci, using both case-control and family study designs. Allelic variation (different versions) in two HLA genes in the MHC class II region (HLA-DRB1 and HLA-DQ1) have been shown to represent the primary genetic determinants of risk for type 1 diabetes, although other class II (HLA-DPB1), as well as class I (e.g., HLA-A, HLA-B) and class III (e.g., TNF) genes may contribute to susceptibility. It has been suggested that genes in the MHC may contribute up to 50 percent of the total genetic risk for type 1 diabetes, although the effect of HLA genes likely represents more than simple increase of risk. Products of the MHC class II genes are centrally important in the immune

response. These proteins bind short peptides derived from foreign or self proteins and "present" them to cells (designated T cells) that coordinate the immune response. If the T cell does not recognize the peptide as coming from a self protein, it initiates an immune response. It is also not clear whether or by how much other genes in the region also affect diabetes susceptibility because the strong effects of the MHC class II genes may overshadow weaker, but still important, contributions of risk by other genes.

Contribution of *INS* to Type 1 Diabetes Susceptibility:

A series of studies has confirmed an association of type 1 diabetes with the insulin gene, *INS*, and in particular, that susceptibility to type 1 diabetes is likely to be directly influenced by the number of repeated elements in the *INS* gene, called the "variable number of tandem repeats region," or VNTR. From studies of European and U.S. families, smaller numbers of repeats (designated class I VNTRs) generally confer increased risk for disease. Larger numbers of VNTRs, designated class III, confer a degree of protection from disease. Interestingly, although humans get a copy of the *INS* gene from each of their parents, it is only necessary for one of those copies to be class III in order to confer resistance to type 1 diabetes. Since the class III VNTRs are associated with higher levels of insulin mRNA in the thymus, it is possible that lower risk of diabetes is associated with higher thymic expression, and thereby higher rates of deletion of self-reactive T cells during development. This possibility has yet to be proven in humans. Furthermore, data from animal models, which show that a portion of the insulin precursor protein is essential for type 1 diabetes in the non-obese diabetic (NOD) mouse, are consistent with the hypothesis that expression of the insulin precursor can directly affect type 1 diabetes incidence in humans.

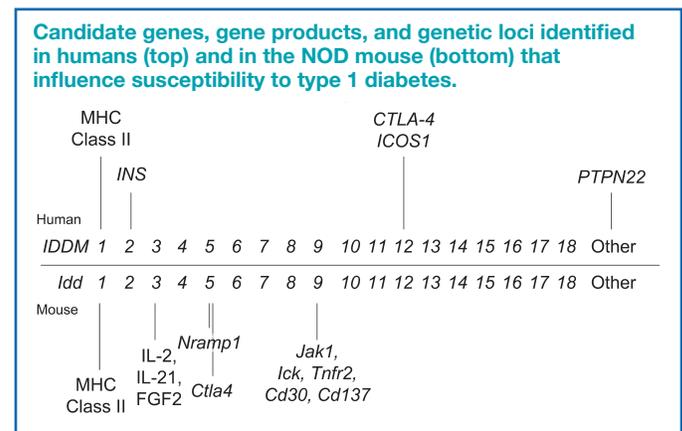## Other Genetic Factors Associated with Susceptibility to Type 1 Diabetes:

Recent studies revealed that the *PTPN22* and *CTLA4* genes also contribute to several autoimmune diseases, including type 1 diabetes. Both genes encode proteins that act as negative regulators of T cell activation. Another gene, *AIRE*, exerts an immune tolerance promoting function by negative selection of T effector cells in the thymus. The absence of the AIRE protein, which results from a rare mutation in people, promotes autoimmunity in several tissues and increases the incidence of type 1 diabetes and other autoimmune diseases.

Exploration of Human Genomic Regions Associated with Type 1 Diabetes Susceptibility:

While candidate genes for type 1 diabetes are the subject of numerous ongoing investigations, there has previously been little coordinated effort to fully explore the regions around these candidate genes or the potential interactions among these genes. Regions of the genome with linkage to, and/or association with, type 1 diabetes include portions of human chromosomes 1p, 2q, 6p, and 11q. The figure below summarizes genes and genetic loci that influence susceptibility to type 1 diabetes. These regions each have a relatively small impact on type 1 diabetes susceptibility, and published studies have insufficient statistical power to precisely quantify their influence on disease susceptibility. To accurately gauge their impact, large numbers of affected sib-pair families, parent-child trios, or case-control collections will need to be studied.

The Type 1 Diabetes Genetics Consortium (T1DGC; www.t1dgc.org) has provided for collection of biological materials required to conduct in-depth genetic studies with sufficient power. The creation of repositories (for DNA, plasma, and serum) for genetic studies provides a resource for research advancement in a cost-effective manner. The ability to discover genes that cause complex diseases has also been greatly facilitated by breakthroughs in large-scale sequencing, genotyping, and data analysis. Continued examination of candidate genes, linkage regions, and application of whole genome association studies, coupled with integration of epidemiologic risk factors identified by other consortia activities, could identify critical pathways that better define an individual's risk of type 1 diabetes.

Candidate genes, gene products, and genetic loci identified in humans (top) and in the NOD mouse (bottom) that influence susceptibility to type 1 diabetes.

Genes and Genetic Concepts Discovered in Animal Models of Type 1 Diabetes:

The NOD mouse and BB rat models are currently the most studied animal models of human type 1 diabetes and show many similarities of disease susceptibility determinants as well as disease process to humans. Most importantly, genetic variability in the peptide-binding pockets of the products of the MHC class II genes—both in humans and in these animal models—is a major determinant of susceptibility to type 1 diabetes. This is

consistent with what is known about the important role for cell-mediated immunity in type 1 diabetes pathogenesis.

Multiple type 1 diabetes susceptibility regions have been identified in NOD mice, some of which have orthologous regions identified in the human genome which could be targets of inquiry in human genetics consortia such as the T1DGC. Further identification of disease-susceptibility regions is in progress via an NIH-sponsored NOD sequencing initiative which compares diabetes-susceptible (NOD) and resistant (B6) mouse strains. Disease genes identified thus far in the NOD mouse model include functional variants encoding beta 2 microglobulin (a component of the MHC protein complex) and *CTLA4*. Further study of newer mouse and rat models of type 1 diabetes, such as the ALR mouse and the LEW.1AR1 iddm rat, will add to this knowledge of gene variants conferring protection from type 1 diabetes, particularly via molecular pathways active within the beta cells themselves. These models provide the means to discover the downstream consequences of disease-associated alleles via molecular and cellular studies. Identification of relevant biological pathways is the basis for targeted preventive strategies and drug development.

An additional role of the NOD mouse model has been the creation of strains of these mice carrying the human class I and II alleles associated with susceptibility to or protection from autoimmune disease. These "transgenic" mice have been used to identify and study T cell antigens potentially involved in the autoimmune response in humans. Similar transgenic approaches to study the function of non-MHC disease genes discovered in human genetic studies will take advantage of similarities between the mouse and human immune systems, including autoimmune disease pathogenesis. Such studies will provide important validation of the role of a human gene or allele in determining type 1 diabetes susceptibility.

Type 1 diabetes in the BB rat is dependent on genetic variants in several loci, including *Iddm2* on chromosome 4, which encodes Gimap5, a gene product that is responsible for the lymphopenia phenotype (reduction in the number of lymphocytes) and is essential to diabetes. This and other genes identified in the rat will provide leads for genes and processes that could be critical determinants of human disease.

Perhaps equally important as the efforts to identify individual genes and genetic pathways affecting diabetes development in various animal models are the efforts to determine the influence of numerous genes and genetic networks on autoimmune disease processes. Mouse and rat models of type 1 diabetes allow investigators to design experiments to measure the consequences of specific gene combinations. Lessons learned from such experiments are critical for improving the modeling of human data in order to reveal gene-gene interactions.

Initiation of Studies To Identify Environmental Causes of Type 1 Diabetes:

Recent evidence suggests that the incidence of type 1 diabetes in children in the United States and several other countries has increased over the last 20 years, particularly in young children and infants. To address the lack of national data on childhood diabetes, the SEARCH consortium is assessing the incidence and prevalence of all forms of diabetes in youth in the United States. Six clinical centers located in California, Colorado, Hawaii, Ohio, South Carolina, and Washington will examine approximately 9,000 children with diabetes to determine the etiology of their disease, including genetic and environmental determinants. This effort will contribute to increased understanding of the pathogenesis of the disease and illuminate the factors underlying the increasing incidence in the United States, which currently is not well understood. Data from children and youth developing diabetes will provide more information to better understand the different types of diabetes currently affecting American children.

Numerous studies have investigated the environmental causes of type 1 diabetes, but have not yielded consistent results. This may be due in part to the failures to account for genetic susceptibility, begin observation at an early age or *in utero*, or monitor patients frequently and long term. The Environmental Determinants of Diabetes in the Young (TEDDY) consortium has developed a comprehensive, multidisciplinary, and rigorous approach to this problem. Researchers in the consortium are establishing a cohort of children with elevated genetic risk for type 1 diabetes by screening newborns in the general population and in families with first-degree relatives diagnosed with type 1 diabetes. This research will lead to better understanding of disease pathogenesis, which provides a foundation for new strategies to prevent, delay, or reverse type 1 diabetes. If the National Children's Study (NCS) were to be implemented to study the effects of environmental influences on the health and development of more than 100,000 children across the United States, it would not have sufficient power to achieve statistically significant results for detecting an association with type 1 diabetes. Therefore, the TEDDY consortium is a unique and necessary effort to identify environmental triggers of type 1 diabetes.

Role of Diet in Type 1 Diabetes: Dietary manipulations in rodent models of type 1 diabetes (BB rat and NOD mouse) affect spontaneous diabetes development. A large international clinical trial, the Trial to Reduce IDDM in the Genetically at Risk (TRIGR), is under way to definitively answer whether early infant exposure to cow's milk increases risk of type 1 diabetes. Large-scale epidemiologic studies have also suggested that early introduction of cereal into the diet may increase the risk of type 1 diabetes. The TEDDY consortium will seek to validate these preliminary findings.

Other factors, such as vitamins B and D, have long been known to modify type 1 diabetes risk in the NOD mouse and the BB rat. Epidemiologic studies indicate that children may be less likely to develop type 1 diabetes when cod liver oil is consumed by the mother in pregnancy, by the infant in the first year of life, or both. A recently initiated feasibility study being performed by the TrialNet consortium, the Nutritional Intervention to Prevent Type 1 Diabetes (NIP), is a pilot study of omega-3 fatty acid supplementation (docosahexaenoic acid) given to pregnant mothers and to newborns at risk before 6 months of age.

Obesity may be a risk modifier for type 1 diabetes. *GAD2* has recently been identified as a strong candidate gene for obesity in certain ethnic populations. *GAD2* is expressed in pancreatic beta cells, and genetic markers called "single nucleotide polymorphisms" (SNPs) in *GAD2* that are associated with obesity also modulate insulin secretion. HLA may have an effect on birth weight, and rapid early growth may increase the risk for type 1 diabetes. Studies in rats suggest that manipulation of the intrauterine milieu by caloric restriction or by low protein diet given throughout gestation affects gene expression in islets and other tissues that may be relevant for beta cell sensitivity to cytokines and toxins in the offspring. Variation within *GAD2* and other genetic factors may be important for the development of islet autoimmunity. These results merit follow-up in longitudinal studies in humans.

Role of Viruses in Type 1 Diabetes: Prospective studies of young children at high risk of developing type 1 diabetes suggest that early and repeated exposure to enteroviruses (e.g., coxsackieviruses) may trigger autoimmunity leading to type 1 diabetes, and that genetically determined host responses to viral infection may influence susceptibility or resistance to the disease. It has been proposed that historical improvements in hygiene may have resulted in decreased immunity to human enteroviruses. The increasing rate of type 1 diabetes in children could therefore be the result of the decrease in humoral protection from enteroviral infections that pregnant women can transfer to their fetuses and mothers can transfer to breast-fed children. For the first time, the TEDDY consortium will test this hypothesis in a standard, prospective manner for enteroviruses and other viruses in several key populations. While large epidemiologic studies have ruled out changes in routine childhood immunizations as a cause of type 1 diabetes, studies such as TEDDY will monitor the effect of changes in immunization program on the risk of autoimmune disease.

Role of Stress in Type 1 Diabetes: Stress has long been considered a potential trigger for type 1 diabetes, and psychological stress may affect the immune system in a variety of complex ways. Recent prospective studies have suggested a link between stress and the development of diabetes-related autoimmunity. In a prospective study of individuals screened for islet cell autoantibodies (ICA), researchers found a greater number of loss experiences during the year before the screening procedure in autoantibody-positive families compared to autoantibody-negative families. Other research has found mothers' experiences of serious life events were associated with increased risk of diabetes-related autoimmunity in their offspring. These results suggest that further investigation is warranted to elucidate the link between environmental stress, the immune system, and type 1 diabetes onset, as is being performed in TEDDY.

RESEARCH OBJECTIVES AND STRATEGIES TO ACHIEVE GOALS

Key objectives for research on type 1 diabetes are to use identified genetic and environmental risk factors to develop interventions to block development of the disease. Research on these mechanisms will provide new insights, not only for type 1 diabetes, but also for other autoimmune diseases, such as thyroid disease, celiac disease, Crohn's disease, rheumatoid arthritis, lupus, and multiple sclerosis. Research on the genes

and variants responsible for susceptibility to type 1 diabetes is facilitated by advances in genetic technology.

Although there has been tremendous progress in genetic and epidemiologic studies of type 1 diabetes, key aspects of the underlying pathogenesis of the disease and its autoimmune process remain unresolved (as discussed in Goal II). Further

research is warranted to identify regions of the genome that harbor type 1 diabetes susceptibility genes, elucidate the genes and their disease-promoting variants, understand the mechanistic functions of the variants, and clarify their interaction with other genes and environmental risk factors and triggers. These research efforts will be important for identification of therapeutic targets and the implementation of molecular medicine strategies for prevention of disease.

The development of several consortia to focus on specific areas of research has been highly productive. Continued progress in understanding the pathogenesis of type 1 diabetes requires collaboration and coordination among geneticists, immunologists, epidemiologists, behavioral scientists, and experts in infectious diseases and nutrition, both across biomedical research sites and Federal and private funding agencies. Hence, further coordination among the consortia, as well as continued support of *ad hoc* working groups, could significantly enhance communication and collaboration.

Many studies, such as T1DGC, TEDDY, the TrialNet Natural History Study, SEARCH, and TRIGR, are accumulating substantial 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. Targeted solicitation would encourage novel applications of genomics, proteomics, and metabolomics to utilize these resources for exploration of aberrant function of genes, proteins, and metabolites for risk of type 1 diabetes.

Genetic Causes

Understanding the genetic basis of type 1 diabetes has been limited by: the number of samples available for analysis; the lack of molecular genetic reagents available to pinpoint susceptibility loci within the human genome; the limitations of analytic and informatics infrastructure available to understand the genetic data; and the inability to functionally characterize potential causative variants in appropriate model systems. Progress in several of these areas provides new opportunities.

The development of genomic technology continues at a rapid pace. The reagents of the Human Genome Project and the HapMap project will permit detailed interrogation of candidate regions and genes that may modulate risk of type 1 diabetes. The HapMap was completed in 2005 and provides detailed knowledge of the variation in the genome, showing the boundaries of neighborhoods of correlated genetic variation, or haplotypes, across the entire human genome. With these

haplotypes defined, HapMap provides an efficient method for choosing "tag SNPs" that capture the genetic variation in each neighborhood with a minimum amount of work.

However, management and analysis of genomic data remain rate-limiting steps in the pursuit of type 1 diabetes susceptibility genes. Further support for genomic capabilities dedicated to type 1 diabetes would permit more comprehensive research to be performed by more investigators. Epigenetic influences on susceptibility to type 1 diabetes should also be investigated. An increased focus on research training and on providing support for the development of cost-effective human DNA sequencing methods and infrastructure for gene-gene and gene-environment analyses should be supported. Because various ethnic groups have different genetic risk factors for type 1 diabetes, pursuit of the genetic basis for these causal variants in multiple populations is a high priority.

Candidate gene studies of type 1 diabetes susceptibility will continue to be informative using both family and case-control designs. Many candidate genes will be identified by general immunology studies, by research on beta cells, and by investigations of animal models of type 1 diabetes, such as the NOD mouse and the BB rat. The effort to discover additional causative genes in animal models of type 1 diabetes will facilitate investigation of the corresponding genes directly in human samples—such as those of the T1DGC—in order to provide insights on the pathways in which the causative genes function.

Research Objective—Create Resources for the Study of Type 1 Diabetes Genetics:

▶ *Complete the Type 1 Diabetes Genetics Consortium (T1DGC)—an unlimited source of DNA for type 1 diabetes gene discovery from informative families representing various ethnic groups.*

The T1DGC is establishing a resource of biological materials that will facilitate research on the genetic basis of type 1 diabetes. In addition, the T1DGC will refine the regions in the genome that contain both MHC and non-MHC type 1 diabetes susceptibility genes using high-throughput linkage disequilibrium mapping methods. The T1DGC will complete a genome-wide scan with the power to detect susceptibility loci with low locus-specific odds ratios, evaluate evidence for gene-gene interactions, and clarify—using appropriately large samples with sufficient power—the effects of hypothesized type 1 diabetes susceptibility loci (e.g., *IL12B*, *SUMO4*) on disease risk.

- *Establish a resource of biological materials that will facilitate research on the genetic basis of type 1 diabetes in those who develop the disease later in life.*

Type 1 diabetes is not only a disease of young people. In fact, up to 50 percent of classical type 1 diabetes (carrying HLA risk alleles and islet cell antibody) may occur after the age of 35 (17). Little information is available about the genetic and environmental causes of type 1 diabetes in patients outside the pediatric population.

Research Objective—Identify Human Genes Causing Type 1 Diabetes:

- *Identify the mechanisms by which the genes within the human MHC contribute to the major genetic susceptibility in type 1 diabetes, and estimate the influence of HLA on other genes with respect to type 1 diabetes risk.*

Genes encoding HLA in the MHC region (described previously) are recognized to be the major genetic risk factors for type 1 diabetes susceptibility because they account for nearly 50 percent of the genetic risk. Yet, the type 1 diabetes susceptibility genes in the MHC have not been fully identified or characterized. Mapping and identification of other loci within the MHC region in Caucasian populations is limited by the extensive linkage disequilibrium in the region and the consequently limited haplotype diversity. In other ethnic groups, the prevalence of type 1 diabetes is much lower, and both disease-associated alleles and patterns of linkage disequilibrium within these populations differ from those observed in Caucasians. These populations offer several significant advantages for mapping and identifying risk variants within the HLA-encoding region, quantitation of allele-specific degree of risk, and completion of fine mapping, both in the HLA portion and in other regions of the MHC where significant evidence of linkage exists.

- *Identify and elucidate the mechanism of non-MHC type 1 diabetes susceptibility loci, and develop, test, and validate appropriate statistical methods for characterizing genome-wide gene-gene interactions.*

Combined, genes other than those in the MHC account for 50 percent or more of the genetic risk for development of type 1 diabetes; however, these genes are likely to have smaller individual effects and may interact with other genes. Thus, there is a need to elucidate the mechanisms whereby the relatively minor (non-MHC) type 1 diabetes susceptibility loci (e.g., *CTLA4*, *PTPN22*) modulate risk of disease. Several loci of relevance may escape identification in linkage analyses of type 1 diabetes families or may be found only in extremely large association studies (e.g., *PTPN22*). Although contributing comparatively minor degrees of risk, such genes could be important as potential drug targets. More such genes may be identified in gene-gene interaction studies and may require the development of new molecular and analytic methods for their discovery. Clusters of interacting genes may facilitate identification of cellular pathways involved in type 1 diabetes pathogenesis and may explain the recognized clinical heterogeneity in disease presentation (differences in age at onset, presence of autoantibodies, and risk for organ-specific complications).

- *Utilize newly developed genomic resources to facilitate testing and cataloging of genomic architecture (SNPs and haplotype blocks) to discover all genes and gene variants affecting susceptibility to type 1 diabetes through a genome-wide association study.*

The Human Genome Project has provided researchers access to the complete sequence of the human genome, greatly facilitating the ability to study the genetics of human diseases. New activities of the Human Genome Project include gene resequencing, SNP discovery, and the HapMap project, enabling new disease-specific projects. Development of DNA sequencing at the individual level will provide genomic data at an enlarged scale previously unimagined; however, improved informatic and analytic procedures will need to be developed to understand these sequence data in light of disease susceptibility.

- *Test in prospective clinical studies which genetic factors affect the development of islet autoimmunity, progression to type 1 diabetes, or both.*

Type 1 diabetes is best predicted by the presence of islet autoantibodies. The majority of autoantibodies are directed against insulin, glutamic acid decarboxylase (GAD65) and IA-2. Autoantibodies against one or several of these autoantigens indicate the presence of islet autoimmunity. Some, but not all, individuals with islet autoimmunity may go on to develop type 1 diabetes. Ongoing prospective studies such as TEDDY, TRIGR, and the TrialNet Natural History Study offer the opportunity to clarify whether genetic factors influence the progression from autoimmunity to type 1 diabetes. Long-term follow-up of these valuable cohorts will be required to address this important question.

Research Objective—Use Knowledge About the Genetic Underpinnings of Type 1 Diabetes To Prevent and Treat the Disease:

- *Integrate knowledge of genetic susceptibility into risk assessment targeted at prevention and treatment of type 1 diabetes.*

Pinpointing those at risk for type 1 diabetes is an essential prerequisite for clinical trials and implementation in the general population of validated preventative approaches. Approximately 90 percent of all type 1 diabetes patients have either the DRB1*03, DQB1*0201 or the DRB1*04, DQB1*0302 haplotype, but many with these genotypes will not develop diabetes. At present, research studies are using only HLA for genetic screening of patients for type 1 diabetes risk. With the knowledge that HLA and non-HLA genes are involved in susceptibility to type 1 diabetes, one can combine HLA with non-HLA genes to better define the type 1 diabetes risk levels. The design of prospective studies such as TEDDY and TRIGR will enable incorporation of this information for evaluation of individual risk prediction of type 1 diabetes in the general population. In these research studies, genetic screening is being performed at birth to identify children at risk for type 1 diabetes, in order to develop a cost-effective and efficient strategy to determine who should be tested for predictive biomarkers (e.g., islet-specific antibodies) periodically throughout childhood. To aid in the identification of novel prevention strategies, it is also necessary to consider gene-environment interactions such that preventative interventions may differ by genotype.

At present, genetic screening for risk assessment is used as a research tool to identify patients eligible to enroll in clinical trials. If effective prevention strategies are identified in the future, the capability to perform screening in the general population will be important so that everyone can benefit from these new strategies. Increased knowledge about the genetic underpinnings of type 1 diabetes could enable the implementation of a public health program of immunogenic screening for pre-type 1 diabetes to eliminate the onset of morbidity and mortality and to enable population-based primary prevention of type 1 diabetes.

▶ *Develop scientifically based methods of communicating risk information.*

Understanding the genetics underlying type 1 diabetes is rapidly outpacing the ability to communicate genetic risk to patients and families. For most diseases, including type 1 diabetes, the role of genetics is complex. High-risk genes suggest that the individual is at increased risk for diabetes, but diabetes onset is not guaranteed. In fact, most individuals with the high-risk genes will never go on to develop diabetes. Screening individuals to identify those with increased genetic risk requires that genetic screening results be presented in a clear and understandable manner. The science of risk communication must proceed in tandem with the science of the genetics

underlying type 1 diabetes. It is also important to assess the impact of risk communication on individuals and families.

▶ *Use genetic information to guide the selection of immunomodulatory treatment in new onset patients and islet transplant recipients.*

Based on the knowledge of disease-associated alleles in human and mouse type 1 diabetes, it is important to determine the function of these genes. This information could spur the development of assays that could be used to identify therapeutic agents directed at the gene product or other steps in pathways involving the gene product. Pharmacogenetic studies could also identify individuals who respond to specific new therapeutic agents and could be utilized in clinical trials of immune modulation. Patients may participate in specific protocols based on a genetic risk profile.

Environmental Causes

During the 1950s and 1960s, the viral disease of measles was widespread. In peak years during that era, 3-4 million cases of measles occurred in the U.S. population, resulting in more than 450 deaths annually (18). Vaccination programs have cut these rates dramatically. In recent years, fewer than 50 Americans developed measles per annum (18). The same strategy has led to the control of rubella, diphtheria, tetanus, and mumps, the near elimination of polio, and the eradication of smallpox. Vaccines provide an excellent example of how prevention is more efficient and effective than treatment as a cure. What sets type 1 diabetes apart from the previously mentioned examples (all of which involve infectious disease with a known disease-inciting agent) is a lack of clear knowledge about which environmental agents promote type 1 diabetes and, until recently, which beta cell autoantigen might be a reasonable target for such an effort. Specifically, with an improved identification of environmental encounters that modulate the processes of beta cell destruction in human type 1 diabetes, and understanding of their interplay with immunoregulatory defects (described in Goal II), potential targets for vaccination should become more obvious.

Previous studies to investigate the environmental causes of type 1 diabetes have yielded inconsistent results, lagging behind studies of genetic causes of the disease, in part due to geographic differences, nonstandardized measures, study design bias, and inadequate sample sizes. The TEDDY study will provide a coordinated, multidisciplinary, and rigorous approach to this problem. Once environmental causes are

definitively identified, strategies for the prevention of type 1 diabetes through clinical trials consortia, such as TrialNet (as described in Goal II), will be much more likely to succeed.

Pathogen detection techniques based upon high-throughput screening for presence of non-human nucleic acids and proteins should be applied to serial samples obtained from children at risk for developing islet autoantibodies and clinical diabetes. It is plausible that one of the well-known and widely prevalent infectious agents (e.g., enterovirus) is responsible. If so, prevention of type 1 diabetes may require eradication of the agent in the population at-large. It is also possible that other viruses may be involved, or that viruses in general may trigger disease onset in genetically susceptible individuals. Once environmental triggers are identified, significant effort will be required to develop vaccines or other prophylactic measures to lower the exposure and prevent disease onset.

Of critical importance is expertise in population-based infectious disease epidemic modeling, including the role of rare viruses (or orphan viruses) in disease acquisition and transmission. Significant advances have recently been made in the area of pathogen detection. These technologies may offer unique opportunities in the search for unknown environmental factors that could trigger the autoimmune process in type 1 diabetes. In any case, understanding the triggering mechanisms, tissue tropism, and trafficking of cells will be important in providing insights into the mechanisms of disease initiation. Generating this knowledge will require attracting experts in these fields to pursue research on type 1 diabetes.

Long-term observational studies that will follow newborns in the general population with high-risk HLA genotypes or newborns who have a first-degree relative with type 1 diabetes are critical to understanding the importance of environmental factors triggering type 1 diabetes.

Research Objective—Monitor Rates of Type 1 Diabetes:

► *Monitor the incidence of type 1 diabetes in a representative sample of the U.S. population, as well as in informative populations around the world, to further define the course, and possibly the causes, of the recent rise in type 1 diabetes.*

Changes in the rate of disease in a population provide important clues about temporal environmental changes that may trigger development of disease. Although the United States has established the SEARCH consortium to assess the incidence and prevalence of all forms of childhood diabetes in six representative regions of the United States, the country lacks immediate nationwide mechanisms to detect type 1 diabetes outbreaks and to monitor incidence rates, unlike other parts of the world that have established childhood diabetes registries.

Research Objective—Assess Environmental Causes of Type 1 Diabetes:

► *Complete enrollment into the TEDDY study, and begin well-powered and nested case-control studies of children enrolled in TEDDY who have developed persistent autoantibodies to GAD65, IA-2, or insulin, in order to systematically evaluate candidate environmental causes of islet autoimmunity.*

The TEDDY study has developed a comprehensive multi-disciplinary and rigorous approach to this problem. Data are being gathered from cohorts of newborns identified as being at genetic risk for type 1 diabetes, both from the general population and from first-degree relatives of patients with type 1 diabetes. These cohorts will be followed for 15 years for the appearance of various beta cell autoantibodies and the development of diabetes. The TEDDY study will document maternal exposures, early childhood diet, reported and measured infections, vaccinations, and psychosocial stresses. Serial samples of serum, plasma, blood cells, mRNA, and stool will be obtained from these children. TEDDY will also establish a central repository of data and biological samples for subsequent hypothesis-based research.

► *Define the effects of intrauterine environmental exposures (e.g., nutrition, stress, infections) on islet development and islet (beta cell) gene expression and function.*
► *Identify molecular genetic mechanisms by which specific environmental agents may trigger islet autoimmunity and promote progression to type 1 diabetes in utero, in early postnatal life, and later in development.*

The TEDDY protocol represents the current best effort at identifying testable hypotheses related to the etiology of type 1 diabetes. While identification of neonates at high genetic risk permits assessment of environmental exposures in infancy and childhood, intrauterine environmental factors may play a key role in setting the stage for diabetic autoimmunity. At present, very little is known about the immunology of pregnancy or about the fetal and neonatal development of the immune system. To address this issue, the TEDDY Clinical Centers will screen and enroll pregnant women whose offspring are likely to be at increased risk because the mother herself, the father, or another child has diabetes. Blood samples from pregnant women will be obtained, and

information on exposure to a potential trigger factor during pregnancy (e.g., an infection, preeclampsia, blood incompatibility, birth weight) will be recorded to elucidate how intra-uterine factors influence their children's risk of developing positive autoantibodies that are markers of type 1 diabetes.

▶ *Explore the possible role of emerging infectious agents, orphan viruses, and intestinal bacteria in the etiology of type 1 diabetes.*

The number of viruses infecting humans is increasing, and viral molecular genetics now permits detection of previously unrecognized infectious agents. However, at present, there is a poor understanding of the mechanisms by which microorganisms colonize the human gut and influence the gut-associated lymphoid system. More effort is needed at a basic level to understand mucosal immunity relevant to autoimmunity, as discussed in Goal II. Further studies are warranted on the close association between type 1 diabetes and celiac disease, as well as the relationship between early exposure to gluten and appearance of islet autoantibodies.

▶ *Translate novel findings about reduced herd immunity through specific vaccination in the general population and relate this to a possible decrease in herd immunity to common viruses such as human enteroviruses.*

Children are now less likely than ever before to suffer from once common infectious diseases. This is due in part to judicious childhood vaccination practices and reduced incidence of human enterovirus infections that were formerly pandemic. Reduced exposure is reducing herd immunity. It has been suggested that exposure to common infections can protect

against autoimmunity. It has been further proposed that type 1 diabetes mimics the polio virus epidemic in which a reduced exposure lowered maternal immunological protection leading to poliomyelitis in less immunologically protected children.

▶ *Explore candidate environmental agents (e.g., food elements, toxins, stress, infectious agents) as triggers for islet autoimmunity and type 1 diabetes in animal models of type 1 diabetes.*

Knowledge about the effects of dietary manipulation, metabolic stress, or viral infection on the development of spontaneous type 1 diabetes in rodent models will be critical for elucidating the molecular mechanisms of environmental triggers, and for developing new therapies to prevent the disease. Thus, a greater understanding of the etiological mechanisms of type 1 diabetes included in this Goal is entwined with the Goal II objective to better understand the regulation of immune responses in type 1 diabetes. Good animal models are a critical means to both ends.

▶ *Establish a resource of biological materials that will facilitate research on the environmental basis of type 1 diabetes in the older population.*

Although long-term observational studies are being carried out in younger populations, little information exists regarding the environmental causes of type 1 diabetes in those who develop the disease later in life. Clearly, such studies will require longer term commitments and broader screening protocols than have yet been employed.