Advances and Emerging Opportunities in Type 1 Diabetes Research: A Strategic Plan

Developed Under the Auspices of the Statutory Diabetes Mellitus Interagency Coordinating Committee

Version 2—For the Scientific Community

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SUMMARY AND RECOMMENDATIONS

The Strategic Plan will serve as a scientific guidepost to the National Institutes of Health (NIH) and to the investigative and lay communities by identifying compelling research opportunities that will inform future type 1 diabetes research efforts and propel research progress on the understanding, prevention, treatment, and cure of type 1 diabetes and its complications for the next decade.

OVERVIEW OF TYPE 1 DIABETES

Type 1 diabetes is a devastating disease in which the body's immune system attacks and destroys insulin-producing beta cells, which are found in clusters in the pancreas called islets. Without this vital hormone, the cells and tissues cannot absorb glucose (sugar), and patients' cells can starve to death, despite high levels of glucose in the bloodstream. Therefore, patients require daily insulin administration for survival. Type 1 diabetes, as patients and parents say, "never takes a day off." Patients or caregivers must constantly monitor glucose levels. If they are too high, patients must take insulin. If too low, they must eat food to boost their glucose levels. The constant burden of this disease greatly affects the quality of life of patients and family members.

Although life-saving, insulin therapy is not a cure. Despite the vigilant efforts of patients to keep their glucose levels as close to normal as possible, chronically high glucose levels (hyperglycemia) damage their organs. This damage, in turn, can result in the development of life-threatening disease complications, such as blindness, kidney failure, nerve damage, lower limb amputation, heart disease, and stroke. These complications can reduce average life span by many years. Given the unremitting demands of diabetes, it is not surprising that it heightens the risks for various psychiatric disorders, such as depression. On the flip side, when patients aggressively manage their glucose levels with insulin therapy to try to prevent these devastating complications, they are at risk for dangerous episodes of low blood glucose (hypoglycemia). Patients may not even be aware that they are experiencing these episodes (hypoglycemia unawareness). If left untreated, hypoglycemia can result in coma and even death. Patients with type 1 diabetes must constantly walk a tightrope to balance the risks of the immediate danger of hypoglycemia and the long-term danger of complications from high blood glucose levels.

Type 1 diabetes differs from type 2 diabetes, which is more commonly diagnosed in adulthood, is strongly associated with overweight and obesity, and disproportionately affects minority populations. However, both forms of the disease share the same complications. Treating diabetes and its complications places an enormous public health burden on the United States.

RESEARCH OBJECTIVES

This Strategic Plan identifies key research objectives that will guide future NIH efforts to achieve six overarching Goals of type 1 diabetes research. The objectives outlined in this Plan build upon recent scientific advances and represent scientific opportunities for overcoming current barriers and achieving progress in type 1 diabetes research over the next 10 years.

Goal I: Identify the Genetic and Environmental Causes of Type 1 Diabetes

Type 1 diabetes results from an interplay of genetic and environmental factors. Several key genes involved in the disease have been identified, but many more remain unknown. Environmental factors have also been found to play a role, but no single trigger has been conclusively identified. Research

on genetic and environmental factors could help predict who will develop type 1 diabetes, and could also lead to the identification of novel prevention strategies. Key research objectives in this area are:

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

Goal II: Prevent or Reverse Type 1 Diabetes

Preventing type 1 diabetes onset would obviate the need for daily insulin administration and the serious disease complications. Research to explore the defects in the immune system that are associated with autoimmunity could lead to new methods to predict, diagnose, treat, and ultimately prevent the disease. In addition, research is required to halt or reverse beta cell destruction after disease onset, to preserve patients' insulin producing capacity. Key research objectives in this area are:

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 the 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

Goal III: Develop Cell Replacement Therapy

Islet transplantation has engendered tremendous hope as a possible cure for type 1 diabetes. This therapeutic strategy replaces the insulin-producing beta cells destroyed by the immune system, thereby eliminating or reducing the need for insulin administration. However, to make this strategy a viable option for most patients, it is imperative to overcome the numerous obstacles that still exist, such as the shortage of available islets and the need for less toxic methods to prevent islet rejection and the recurrence of autoimmunity. Research on both beta cell biology and clinical islet transplantation can help to overcome these and other barriers. Key research objectives in this area are:

Islet Transplantation

- Develop Novel Strategies and Infrastructure That Support Advancing Pancreas Procurement and Islet Processing
- Develop Improved Methods To Assess Islet Beta Cell Viability and Function That Predict Early Islet Function After Transplant
- Investigate the Use of Porcine Islets as an Alternate Source of Islets for Transplantation
- Improve Islet Transplant Procedures
- Develop Novel Methods To Accurately Assess the Post-Transplant Islet Mass
- Harness New Understanding of the Immune System To Develop Improved Clinical Monitoring and Immunotherapies

Pancreatic Development, Stem Cells, and Regeneration

- Grow a Renewable Supply of Pancreatic Beta Cells That Can Be Transplanted into Patients
- Understand How Mature Beta Cells Are Maintained and Replenished in the Adult Pancreas
- Develop Strategies To Regenerate Beta Cells Through Replication or Neogenesis

Goal IV: Prevent or Reduce Hypoglycemia in Type 1 Diabetes

Hypoglycemia is a distressing, acute complication of type 1 diabetes. Low blood glucose impairs brain and other bodily functions, including defenses against future hypoglycemia episodes, causing a vicious cycle of recurrent events. Understanding how the brain and body work together to sense and

adjust glucose levels, as well as research to improve and link glucose monitoring and insulin delivery, could help scientists develop strategies to prevent hypoglycemic episodes and improve patients' quality of life. Key research objectives in this area are:

Brain and Peripheral Nervous System Mechanisms of Hypoglycemia

- Define the Mechanisms and Modulators of Metabolic Sensing
- Elucidate Brain Alterations in Response to Hypoglycemia
- Develop New Strategies To Prevent or Reverse Hypoglycemia-Associated Autonomic Failure

Clinical Interventions To Prevent or Reduce Hypoglycemia

- Control Hypoglycemia Through Behavioral Therapies
- Close the Loop: Develop the Tools Required for an Artificial Pancreas

Goal V: Prevent or Reduce the Complications of Type 1 Diabetes

Persistent elevation of blood glucose can lead to life-threatening disease complications. Research has dramatically demonstrated that intensive control of blood glucose levels can prevent or delay the development of these complications. However, because of the limitations and difficulties of current therapies for achieving good glucose control, as well as the threat of hypoglycemia associated with intensive control, patients rarely achieve recommended glucose levels. Future research strategies will build upon the existing approaches to control diabetes, as well as develop novel approaches to break the link between high glucose and chronic complications. Key research objectives in this area are:

Molecular Mechanisms of Common Pathways in Diabetic Complications

- Identify Molecular Pathways of Hyperglycemia Damage
- Clarify Mechanisms Linking Fuel Utilization and Heart Disease
- Understand the Systems Biology of Diabetic Complications

Metabolic Memory

- Discover the Molecular Mechanisms of Metabolic Memory

Genetic Factors

- Identify Genes Conferring Susceptibility and Resistance to Diabetic Complications

Animal Models

- Develop More Human-like Animal Models of Diabetic Complications

Biomarkers and Surrogate Endpoints To Facilitate Clinical Trials

- Identify Biomarkers or a Combination of Biomarkers for Earlier Detection of Cell and Tissue Damage
- Validate Surrogate Endpoints for Assessing the Progression of Complications in Clinical Trials

Therapies To Improve Patient Health

- Identify Therapeutics That Prevent or Reverse the Development and Progression of Diabetic Complications
- Mitigate Psychosocial Complications and Comorbidities of Diabetes To Improve Quality of Life
- Combine New Technology for Diabetes Management with Behavioral and Translational Research

Goal VI: Attract New Talent and Apply New Technologies to Research on Type 1 Diabetes

Continued research progress depends on attracting and training a workforce of scientists with diverse expertise to conduct research on type 1 diabetes and its complications. In addition, the harnessing of new and emerging technologies sets the stage for innovative discoveries that can bring tremendous benefits to patients. Key research objectives in this area are:

Engaging Talented Scientists

- Recruit Expertise from Diverse Fields
- Design Incentives That Reward Research Innovation
- Train New Scientists in Clinical Type 1 Diabetes Research

Development and Application of New Technologies

- Develop Noninvasive Imaging Technologies To Monitor Type 1 Diabetes
- Promote Application of Advances in Bioengineering to Type 1 Diabetes
- Foster Application of Gene Delivery and Gene Silencing Technology To Develop New Therapies for Type 1 Diabetes and Its Complications
- Apply New and Emerging Technologies in Functional Genomics, Proteomics, and Metabolomics to Type 1 Diabetes Research
- Improve the Power of Diabetes Research by Utilizing Computational Biology and Bioinformatics
- Apply New Technology to the Development of Improved Animal Models for the Study of Type 1 Diabetes

NIH SUPPORT FOR TYPE 1 DIABETES RESEARCH

Research toward achieving the six overarching Goals has been accelerated by the *Special Statutory Funding Program for Type 1 Diabetes Research*. The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) administers this special appropriation on behalf of the Secretary of the Department of Health and Human Services (HHS), in collaboration with multiple other NIH Institutes and Centers, and the Centers for Disease Control and Prevention (CDC). The *Special Funding Program* has allowed the creation of unique, collaborative, and innovative research consortia and clinical trials networks to increase understanding about the prevention, treatment, and cure of type 1 diabetes. Initiatives supported by the program are different in size, scope, duration, and nature from other type 1 diabetes efforts supported through regular NIH appropriations. The *Special Funding Program* enabled the initiation of most of these large-scale, high-impact efforts, at a scientifically optimal scale of operation. Importantly, the research efforts that have been supported to date have spurred numerous future opportunities that could dramatically improve the lives of patients with type 1 diabetes. Type 1 diabetes research is also supported by regularly appropriated funds to HHS.

IMPLEMENTATION: GUIDING FUTURE RESEARCH EFFORTS

This Strategic Plan reflects a dynamic planning process that involves collaboration among numerous stakeholders to ensure that research progress is regularly assessed and that new and emerging opportunities are identified. The statutory Diabetes Mellitus Interagency Coordinating Committee will continue to play a key role by assessing progress toward attaining the goals and objectives described in this Plan, which was developed under its auspices. The NIH will also continue to solicit broad external input from the scientific, lay, and patient advocacy communities to inform its planning efforts. The NIH will use the research objectives described in this Strategic Plan as a scientific guidepost to improve current treatment strategies and to identify ways to prevent or cure type 1 diabetes and its complications.

Process for the Development of the Type 1 Diabetes Research Strategic Plan

Origin

One of the recommendations emanating from a January 2005 *ad hoc* planning and evaluation meeting focused on large scale efforts made possible by the *Special Statutory Funding Program for Type 1 Diabetes Research* was that the NIH should initiate a broad review of the entire state-of-the-science with respect to type 1 diabetes and its complications. In response to this recommendation, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) launched a new strategic planning effort for type 1 diabetes research.

Collaborative Planning Process

This Strategic Plan was developed through an open and inclusive planning process, with oversight by the statutory Diabetes Mellitus Interagency Coordinating Committee, and leadership by the NIDDK Division of Diabetes, Endocrinology, and Metabolic Diseases. The Committee, chaired by the NIDDK, includes representation from NIH components involved in diabetes research, as well as from other relevant federal agencies.

To develop the scientific chapters of the Strategic Plan, Working Groups were convened to identify recent scientific advances and research objectives for Goals I-V. Goal VI, "Attract New Talent and Apply New Technologies to Research on Type 1 Diabetes," was addressed by all Working Groups because it is an interdisciplinary goal that applies across type 1 diabetes research. The Working Groups were composed of a diverse and talented group of individuals who are committed to propelling progress in type 1 diabetes research. They were chaired by scientists external to the NIH, with membership that included extramural scientists, NIH representatives, patients, and representatives from patient advocacy groups.

Public comment was solicited prior to publication by the posting of the draft plan on a Web site created for the planning effort (www.T1Diabetes.nih.gov/plan).

Organization of the Strategic Plan

The Strategic Plan was framed around the six overarching scientific goals of type 1 diabetes research. One version of the Plan was developed for patients with type 1 diabetes, their family members, and the public. It contains a description of how research addressing each goal could benefit people living with type 1 diabetes and their family members, as well as profiles of patients and scientists. Another version of the Plan was developed for the scientific research community. While tailored to different readers, both versions highlight key recent scientific advances that have accelerated research and/or benefited patients' health, and identify the most compelling opportunities and objectives for research.

Both versions of the Plan contain a summary of major research objectives. Research objectives are specific research directions that can be pursued over the next decade, within available NIH resources, to realize the goal of each chapter. In some cases, these objectives intersect with one another and may be dependent upon one another for progress. For example, identifying environmental triggers of type 1 diabetes (Goal I) will help to inform future disease prevention strategies (Goal II). Also, "Attract New Talent and Apply New Technologies" (Goal VI) is important for every area of type 1 diabetes research. The Strategic Plan describes a coordinated, multifaceted approach for significantly advancing research to combat type 1 diabetes.

INTRODUCTION

OVERVIEW, BURDEN, AND IMPACT OF DISEASE

This Strategic Plan focuses on type 1 diabetes—the form of the disease in which the body's immune system destroys the cells that produce insulin, a hormone that regulates the amount of glucose (sugar) in the blood and is essential for life. Because patients with type 1 diabetes no longer produce insulin, which is necessary for survival, they require daily insulin administration, either through injections or an insulin pump. In the other major form of diabetes—type 2 diabetes—loss of effective insulin action is due to a combination of defects, both in normal insulin action (insulin resistance) and in the ability of pancreatic beta cells to overcome this insulin resistance by secreting sufficient amounts of additional insulin. Both forms of the disease share the same possible complications, which include blindness, kidney failure, nerve damage, lower limb amputations, heart disease, and stroke.

Type 1 diabetes can be more serious and costly for patients because it tends to strike earlier in life. For example, while type 2 diabetes increases the risk of heart disease 2- to 4-fold (1), heart disease risk is increased by up to 10-fold in patients with type 1 diabetes compared to the general age-matched population (2, 3). Importantly, the longer a person has diabetic complications, the more severe, difficult-to-treat, and costly they can become. Thus, an early diagnosis of type 1 diabetes can set the stage for a lifetime of living with and medically managing the disease complications. Few chronic medical conditions rival type 1 diabetes in terms of the extent to which maintenance of acceptable health is so heavily dependent on the capacity of patients and families to make and execute effective self-management decisions while simultaneously addressing many other complex priorities.

With respect to quality of life, dreaded complications can diminish the vitality of childhood and adolescence, as well as the prime productivity of young adulthood. Patients and their parents often wait anxiously to receive test results of their eye and kidney function. A broken blood vessel in the retina, or the finding of protein in the urine, can be the first sign that a relentless complication of the disease has emerged, and that grueling and costly treatments are in the near future. Even with recent advances in treatment, type 1 diabetes is estimated to lower average life expectancy by 15 years (4). For childhood-onset cases, greater than 15 percent of patients with type 1 diabetes will die by age 40 (4). Thus, early onset type 1 diabetes has major adverse impacts on patients and on society because of its extremely high personal and economic costs.

Type 1 diabetes has much in common with type 2 diabetes despite key differences in the mechanisms underlying development of the two forms of diabetes. Both involve malfunctions in the body's system for maintaining appropriate blood glucose levels due to defects in insulin production. Thus, research to understand the intricacies of insulin-producing beta cells, and to find ways to preserve and restore beta cell function, would benefit all diabetes patients. Similarly, the mechanisms of hypoglycemia (dangerous episodes of low blood sugar that can lead to coma and death) are also common to both forms of the disease and limit the ability to deliver therapy proven to prevent or slow complications. Therefore, research to understand and counteract hypoglycemia's effects on the brain would help those with both forms of diabetes. In the same way, all diabetes patients would gain from research directed toward understanding, treating, and preventing the eye, nerve, kidney, heart, and other complications that type 1 and type 2 diabetes share. Alarmingly, both forms of the disease are being increasingly diagnosed at a younger age,

1901

"Diabetes Mellitus" defined as "destruction of the islands of Langerhans."

1915

"Rainbow test," glucose monitoring with Benedict's Solution, provided an inexpensive way to roughly measure sugar levels in urine.

1921

Frederick Banting and Charles Best "discover" insulin, successfully treating a diabetic dog.

1922

First diabetes patient successfully treated with insulin.

1935

Doctors recognized distinction between type 1 and type 2 diabetes based on "insulin sensitivity."

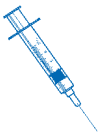
when the disease is more difficult to control; earlier onset increases diabetes' toll in lost health and productivity. Research aimed at diabetes in pediatric populations may help to shed light on and combat this trend. Furthermore, researchers are increasingly recognizing that many patients may have "hybrid" forms of diabetes. Careful characterization of patients considered to have type 2 diabetes reveals that a subset also have markers of type 1 diabetes known as autoantibodies. Interestingly, some patients with type 1 diabetes have the "insulin resistance" that was previously considered a hallmark of type 2 diabetes. Over the past 10 years, evidence has mounted to show that, in type 1 diabetes, high blood glucose levels themselves eventually cause secondary insulin resistance in nearly all patients. These observations are further blurring the line that has historically separated the two forms of the disease. They underscore how research progress on one form of the disease can have enormous benefits for the other form as well.

The interdependence and synergism of research on the two forms of diabetes have been clearly demonstrated, and research on type 1 diabetes has already contributed greatly to improved management of both forms of the disease. For example, a landmark clinical trial in type 1 diabetes, the Diabetes Control and Complications Trial (DCCT), proved that intensive glucose control can prevent or delay damage to the small blood vessels in the eyes, kidneys, and nerves (microvascular complications). The findings of this trial paved the way to studies that replicated these impressive results in patients with type 2 diabetes. Most recently, the DCCT findings were extended to show that intensive control reduces heart attacks and strokes (macrovascular complications), the major cause of death in both forms of diabetes. Because of this pioneering research in type 1 diabetes, close control of blood glucose levels is now a cornerstone of the medical management of both forms of the disease. Moreover, this landmark trial in type 1 diabetes also established the value of hemoglobin A1c (HbA1c) levels—a measurement of blood glucose levels over time—as an outcome measure for future clinical trials in both type 1 and type 2 diabetes, dramatically shortening the cost and duration of clinical trials of new therapies and encouraging development of new therapies for diabetes. The use of HbA1c as an outcome measure was the basis for Food and Drug Administration (FDA) approval of improved forms of injected insulin, inhaled insulin, and several new classes of oral drugs for type 2 diabetes, which when used in combination can delay the need for insulin therapy.

Unfortunately, most national data on the incidence, prevalence, and burden of diabetes do not distinguish between type 1 and type 2 diabetes—although ongoing studies may help to address this problem. Within the context of available data, it is generally estimated that type 1 diabetes accounts for 5 to 10 percent of all diagnosed cases of diabetes in the U.S. (5). However, the burden of type 1 diabetes is disproportionate to its prevalence because complications and loss of quality life years are much greater when diabetes strikes at younger ages. Collectively, both type 1 and type 2 diabetes constitute an enormous public health challenge in the United States. Although this Strategic Plan is focused on type 1 diabetes, the following indicators of the overall burden of diabetes are presented because the best available epidemiological data are reported for diabetes as a whole.

- Patients with diabetes have an increased risk of heart disease and heart attacks, stroke, high blood pressure, kidney failure, blindness, nerve pain and other neurological problems, limb amputation, chronic wounds and skin ulcers, periodontal disease, depression, and pregnancy-related problems.
- In the past 25 years, the number of people with diabetes has more than doubled to 20.8 million (5, 6), or 7 percent of the total U.S. population (5). Evidence now suggests that one in three Americans born in 2000 will develop diabetes during his or her lifetime (6). It is generally believed that these trends are largely attributable to type 2 diabetes, and related to increases in obesity, a known risk factor for type 2 diabetes, as well as to changes in demographics, such as increases in the elderly and minority populations of the United States who are prone to developing type 2 diabetes. Rates of type 1 diabetes are increasing in some European countries where reliable data are available.

1944



Standard insulin syringe is developed, helping to make diabetes management more uniform.

1948

Development of delayed action insulin reduced the number of daily insulin injections.

1950

National Institute of Diabetes & Digestive & Kidney Diseases

Medical Research Act established national research institute for diabetes, which later became NIDDK.

1950

Discovery that insulin promotes glucose transport.

1955

Ile-Val-Glu-Gln-Cys-Cys-Ala-Ser-Val-Cys-Ser-Leu-Tyr-Gln-Leu-Glu-Asn-Try-Cys-Asn

Amino acid sequence of insulin protein determined.

More limited data suggest that rates of type 1 diabetes are increasing in very young children and infants in the U.S. Baseline national data on diabetes in children have recently been collected in the U.S., and the next phase of this study will provide definitive information on whether type 1 diabetes rates in children are stable or changing.

- Diabetes is the sixth leading cause of death in the United States, resulting in more than 73,000 deaths in 2002 (7). More than 224,000 people die annually from diabetes-related complications common to both type 1 and type 2 diabetes. This number is considered to be significantly underreported (5).
- The problem of diabetes extends globally. The World Health Organization estimated that 1,125,000 people worldwide would die from diabetes in 2005 (8). Overall, the risk of death for individuals with diabetes is approximately double that of people without diabetes of similar age (5).

The burden of both forms of diabetes extends far beyond mortality. In the United States each year, 12,000 to 24,000 people become blind as a result of diabetic eye disease and approximately 82,000 people undergo diabetes-related amputations (5). Encouragingly, declines in the incidence of end-stage renal disease (ESRD) due to diabetes are being noted for the U.S. population, in reports from the United States Renal Data System. These improvements are most noteworthy in patients under age 30 with diabetes (most of whom have type 1 diabetes) and have been observed in Caucasians, but not in African Americans (9). However, ESRD remains a major public health problem. In 2003, 45,330 Americans with diabetes began treatment for irreversible kidney failure (ESRD), and 165,113 people with failed kidneys needed chronic dialysis or a kidney transplant to remain alive (9).

The financial burden of diabetes is tremendous. The direct and indirect costs associated with both forms of diabetes in the United States during 2002 were estimated to be $132 billion (5). The average annual health care costs for a person with diabetes are $13,243, which is 2.4 times greater than those for an individual without diabetes (10). In 2002, 11 percent of national health care expenditures were directed to diabetes care (10). The costs of treating the complications of diabetes, which both forms of the disease share in common, account for much of the health care costs associated with the disease. Although estimates of the rates of diabetes have increased since 2002, the associated cost estimates have not yet been revised; hence, the economic data given here are conservative. Clearly, the economic and societal burden of diabetes has a profound impact on the Nation.

Incidence and Prevalence of Type 1 Diabetes

In the United States, it is estimated that approximately 1 in every 400 to 600 children and adolescents has type 1 diabetes (5). There is evidence that the incidence (the number of new cases) and prevalence (the total number of cases) of the disease are increasing in Europe. In the United States, the incidence and prevalence of type 1 diabetes are not precisely known because of the lack of uniform national data on the rates of childhood diabetes and how the rates are changing over time. This gap in knowledge is being addressed by the Search for Diabetes in Youth Study (SEARCH), which is determining the prevalence and incidence of diabetes in children and youth less than 20 years of age. Emerging data from the SEARCH study (11) suggest that the incidence of type 1 diabetes in American children may be higher than an earlier estimate of 13,000 per year (12). In total, about 30,000 people (children and adults) are diagnosed with type 1 diabetes annually (12).

Key Features of Type 1 Diabetes

Type 1 diabetes is an autoimmune disease in which the body's own immune system attacks and destroys specialized cells of the pancreas called beta cells. Beta cells are found within tiny clusters called islets and produce the hormone insulin. Insulin is required for survival; it sends signals to the body's cells and tissues, telling them to absorb glucose to use as a fuel. Without this vital hormone, the cells and tissues do not absorb glucose and patients can starve to death, despite having high levels of glucose in their bloodstream. An interplay of

1956

Development of the "dip-and-read" urine test allowed instant monitoring of glucose levels.

1959

Immunoassay allowed researchers to measure insulin in blood. This assay showed that patients with type 1 diabetes produced no insulin, but patients early in the course of type 2 diabetes had more insulin than normal.

1960

Self-monitoring of blood sugar with the "wet" method using glucose oxidase strips.

1964

First kidney transplants in patients with diabetes.

1966

First successful pancreas transplant performed.

genetic and environmental factors is responsible for the onset of type 1 diabetes (as well as type 2 diabetes). Having a family member with the disease puts one at higher risk for developing type 1 diabetes.

Type 1 diabetes differs from type 2 diabetes—which is more commonly diagnosed in adulthood, is strongly associated with overweight and obesity, and disproportionately affects minority populations. Although patients with type 1 diabetes require externally administered insulin to survive, type 2 diabetes patients may be treated with medications that make their tissues more sensitive to insulin or enhance insulin production or, in some cases, may be treated with insulin itself.

Treatment Options and Challenges

The treatment of patients with type 1 diabetes was revolutionized in 1921 with the discovery of insulin by a group of researchers at the University of Toronto. To this day, insulin therapy continues to save the lives of patients with type 1 diabetes by replacing the essential hormone that their bodies no longer adequately produce. However, insulin therapy, whether through injections or via a pump, is not a cure and it cannot prevent complications. To manage the disease, patients must carefully monitor their food intake and physical activity. They must perform painful finger sticks multiple times a day to draw blood and test their glucose levels. Based on this monitoring, patients often give themselves several shots of insulin a day, or calculate the correct amount of insulin to administer through their insulin delivery pumps. This regimen is not just "once in a while;" it is every day of their lives. As many patients and their parents say: "There is never a day off from diabetes." Moreover, no matter how vigilant patients are at regulating their blood glucose levels, they can never achieve the fine-tuned regulation provided by a healthy pancreas, which exquisitely senses and responds to insulin needs with precise timing.

Because of the inadequacies of insulin treatment, patients with type 1 diabetes are susceptible to harmful fluctuations in their blood glucose levels—abnormally high blood glucose (hyperglycemia) or dangerously low blood glucose (hypoglycemia). Both of these conditions can be life threatening in extreme cases. In the case of a very young type 1 diabetes patient who cannot self-monitor, parents must assume the role that is no longer performed by the pancreas. The psychosocial impact on families is enormous. Parents often forego restful sleep because they are "on watch" to ensure that their child's blood glucose levels do not fall dangerously low in the middle of the night. They are also dependent on an extended team of caregivers when their child is not in their immediate care, such as school personnel, childcare providers, friends, and parents of their child's friends.

Approaches for Preventing or Reversing the Disease

Currently, there are no known methods to prevent type 1 diabetes. However, recent clinical trials suggest that it may be possible to reverse or slow the rate of loss of the insulin-producing beta cells in newly diagnosed patients. While the environmental factors that may play a role in triggering type 1 diabetes remain to be defined, several key genes that increase the risk of type 1 diabetes have been identified. Genetic tests in combination with blood tests to detect antibodies directed against the insulin-producing beta cells can predict development of type 1 diabetes, allowing new strategies for prevention to be tested. Key strategies for preventing much of the burden of the disease include early detection, improved methods and delivery of care, and new interventions.

With the number of individuals with diabetes increasing, the associated societal and economic burdens will continue to rise. Yet, there are many positive developments, including reports showing that life expectancy for patients with type 1 diabetes is increasing (13). A key finding of NIH-supported research is that intensive control of blood glucose levels can dramatically prevent or delay the development of disease complications. Now, it is essential to find more effective ways to achieve blood glucose control. Progress being made in

1967

Laser treatment revolutionized the care of diabetic retinopathy.

1969

Determination of the three-dimensional protein structure of insulin.

1971

NIH scientists discover the insulin receptor: a protein on the cell surface that mediates effects of insulin in cells.

1974

Evidence that type 1 diabetes is an autoimmune disease provided by discovery of antibodies to insulin-producing cells in newly diagnosed patients and by genetic studies showing the association of type 1 diabetes with the HLA genes that control the immune system.

1977

Introduction of insulin pumps for continuous delivery of insulin.

the area of cell-based research could lead to ways to replace or restore a patient's insulin-producing capacity. Increased knowledge about the underlying mechanisms of beta cell development and function could potentially be used to develop therapeutic approaches to reverse the disease by promoting formation of new beta cells in the pancreas.

With continued, vigorous research, new strategies may be developed to prevent type 1 diabetes in those at risk, restore insulin independence in patients already diagnosed, and prevent the development of disease complications. Through research in these and other avenues, the burden of type 1 diabetes on people and the Nation can be lifted.

GOALS OF TYPE 1 DIABETES RESEARCH

The promise of a cure for type 1 diabetes can only be realized through the vigorous support of scientific research. Type 1 diabetes research supported by the NIH is focused around six overarching research Goals listed below. Pursuit of research toward attaining each of these broad, scientific Goals can help achieve real progress in the understanding, prevention, treatment, and cure of type 1 diabetes and its complications. The Goals are interdependent in that research on one Goal will inform research on others. Therefore, to maximize research progress, research toward achieving the Goals requires well-coordinated and integrated efforts, as described in this Strategic Plan.

Six Overarching Goals of NIH-Supported Type 1 Diabetes Research

- **Goal I: Identify the Genetic and Environmental Causes of Type 1 Diabetes**
- **Goal II: Prevent or Reverse Type 1 Diabetes**
- **Goal III: Develop Cell Replacement Therapy**
- **Goal IV: Prevent or Reduce Hypoglycemia in Type 1 Diabetes**
- **Goal V: Prevent or Reduce the Complications of Type 1 Diabetes**
- **Goal VI: Attract New Talent and Apply New Technologies to Research on Type 1 Diabetes**

Goal I: Identify the Genetic and Environmental Causes of Type 1 Diabetes

Type 1 diabetes has a strong genetic basis that is modified by environmental factors. It is a "polygenic" disease, which means that it arises from the interaction of variations in multiple genes. Research has already identified some genes that are important in the development of type 1 diabetes. However, researchers have not yet found all of the genes that can play a role in disease development. Identification of key genes will not only help to predict who will develop the disease, but will also aid in the development of new prevention strategies. In addition to genes, the environment has also been found to play an important role in the development of type 1 diabetes. Potential environmental triggers are thought to include viruses, dietary factors, environmental toxins, and psychological stress. To date, no single trigger has been conclusively identified. Research to identify the key environmental trigger(s) could be used to prevent the disease in genetically susceptible people.

Goal II: Prevent or Reverse Type 1 Diabetes

One obvious way to attack type 1 diabetes is to stop it before it starts. Preventing the disease means that patients would not require insulin administration or develop life-threatening disease complications. While recent clinical trials have suggested that further loss of insulin production can be slowed

1978

Development of glycosylated hemoglobin (HbA1c) test permitted monitoring average blood glucose control over a 90-day period.

1978

Insulin gene becomes first human therapeutic protein to be cloned and synthesized by genetic engineering.

1980

Development of first animal model of type 1 diabetes that could be used to test drugs for type 1 diabetes: non-obese diabetic (NOD) mouse.

1983

Introduction of the first biosynthetic human insulin.

1983

Clinical studies showed that preconception care of women with diabetes dramatically reduced congenital malformations in their babies.

in patients with newly diagnosed type 1 diabetes, research has not yet identified an effective disease prevention strategy. However, the ability to identify at-risk individuals permits promising strategies for prevention to be tested in rigorously designed clinical trials. Further research and increasing knowledge about what goes wrong with the immune system will facilitate the discovery of novel ways to prevent autoimmunity, and thus prevent disease onset.

In addition to preventing the disease before beta cell destruction starts, it is important to conduct research to prevent further beta cell destruction in newly diagnosed patients. Research has shown that, after patients are diagnosed with the disease, they still have some beta cell function and can produce C-peptide, a by-product of insulin production which is co-secreted from the beta cell with insulin and is a useful measure of endogenous insulin production. Furthermore, clinical studies have demonstrated major benefits of residual beta cell function in patients with type 1 diabetes, even though the patients require insulin therapy. For example, the DCCT demonstrated that higher and sustained levels of C-peptide were associated with reduced incidence of long-term disease complications of the kidneys and the eyes, as well as reduced hypoglycemia. This evidence suggests that preserving patients' remaining beta cell function could have dramatic, long-term health benefits. Already one agent has been shown to preserve beta cell function in new onset type 1 diabetes. To prevent or reverse beta cell destruction in newly diagnosed patients, further research efforts are required to identify and test additional strategies that may provide more durable benefits and few side effects.

Goal III: Develop Cell Replacement Therapy

Patients with type 1 diabetes require insulin therapy because their immune systems have destroyed their pancreatic beta cells. A real "cure" for this disease could be achieved by replacing those missing cells, and scientists are aggressively pursuing this avenue of research. A major breakthrough occurred in 2000 when researchers at the University of Alberta in Edmonton, Canada, reported that patients with type 1 diabetes achieved insulin independence after transplantation with islets from two to four donor pancreata and treatment with a novel immunosuppressive regimen that omitted the widely used glucocorticoid drugs that are toxic to islets. A major barrier limiting the widespread use of islet transplantation is the shortage of islets available for transplantation. The diabetes research community believes that there is significant potential in the use of human embryonic[1] and tissue-specific adult multipotent progenitor cells in deriving a host of differentiated cell types, including insulin-producing beta cells. Understanding the underlying molecular mechanisms of beta cell biology, and how mature beta cells are formed from stem/progenitor cells, could help to overcome this barrier. Furthermore, as noted previously, recent research has shown that patients with type 1 diabetes have some remaining functional beta cells. Therefore, research on the mechanisms controlling islet cell growth and regeneration could lead to novel therapies designed to stimulate beta cell growth *in vivo*.

Another major barrier that prevents islet transplantation from being a widespread treatment option for patients with type 1 diabetes is the need for lifelong immunosuppressive drug treatments that are currently required to prevent rejection of transplanted islets. Research to identify ways to overcome the need for immunosuppressive treatment, or to identify less toxic immunosuppressives, can help to make islet transplantation a reality for greater numbers of patients with type 1 diabetes.

Goal IV: Prevent or Reduce Hypoglycemia in Type 1 Diabetes

Hypoglycemia is perhaps the most distressing acute complication of type 1 diabetes. Hypoglycemia can occur with missed meals, during exercise, or when too much insulin is in the body, which causes glucose to fall to dangerously low levels. Too little glucose means that the body—and particularly the brain—cannot function at its normal capacity. The immediate effects of hypoglycemia can include changes in cardiovascular and nervous system function, cognitive

[1] The NIH supports human embryonic stem cell research consistent with federal funding policies.

1984

Identification of early kidney disease marker—microalbuminuria—permitted doctors to intervene to prevent or delay kidney failure.

1987

Diabetic foot ulcers treated by total contact casting.

1990

First successful transplant of human islet cells reversed insulin dependency in patients.

1993

Diabetes Control and Complications Trial (DCCT) demonstrated that intensive therapy delays the progression of long-term complications of the eyes, kidneys, and nerves.

1993

Collaborative study of diabetic nephropathy showed that ACE inhibitors reduce need for kidney dialysis and transplantation, and cut heart disease mortality.

impairment, increased risk for unintentional injury, coma, and sometimes death. In some cases, patients are not aware that their blood glucose level is dangerously low. This syndrome is called "hypoglycemia unawareness." It is characterized by the loss of the warning symptoms that alert patients that it is time to eat before their blood glucose level falls too low. In addition, episodes of hypoglycemia impair defenses against future hypoglycemia, resulting in a vicious cycle of recurrent episodes.

A severe limitation to the practice of intensive glucose control to prevent disease complications is the potential for acute episodes of hypoglycemia. It is estimated that patients on intensive treatment have two hypoglycemic episodes a week versus one episode if they are treated less intensively (14). Because intensive glucose control dramatically reduces the risk of long-term disease complications, it is imperative to pursue research to overcome this major obstacle to achieving tight glucose control. The risk of severe hypoglycemia may be related to a variety of behavioral and psychological variables, and behavioral interventions may reduce these risks. Strategies to meld technological, behavioral, and educational advances are key to this goal. Devices for minimally invasive continuous glucose monitoring, developed with NIH support and recently approved by the FDA, may represent a major advance in this regard. Further research is needed to improve glucose monitoring, to link monitoring devices to insulin delivery, and to empower patients and care providers to maximize the benefits of these devices. By reducing hypoglycemic episodes, improving glycemic control, and lessening the burdens of diabetes self-management, this research can also have a major impact on patients' quality of life until cell replacement therapy becomes a viable option for patients with type 1 diabetes.

Goal V: Prevent or Reduce the Complications of Type 1 Diabetes

Nearly every organ in the body is adversely affected by type 1 diabetes. Throughout the course of a patient's life, the persistent elevation in blood glucose levels despite insulin therapy damages vital organs, including the heart and kidneys. The longer a person has the disease, the more likely it is that he or she will develop these severe complications. Because patients with type 1 diabetes are often diagnosed in childhood and adolescence, they may develop complications at a young age.

The DCCT reported good news regarding preventing or delaying the onset of complications. Completed in 1993, the trial compared the relationship between intensive versus conventional treatment of blood glucose levels and the development of disease complications. The DCCT proved conclusively that intensive therapy reduces the risk of microvascular complications, such as diabetic eye, kidney, and nerve disease. Nearly all patients who participated in the DCCT volunteered to continue to be followed in the Epidemiology of Diabetes Interventions and Complications Study (EDIC), which began in 1994. The DCCT/EDIC researchers continue to report remarkable long-term benefits of intensive blood glucose control in preventing or delaying complications of the eyes, kidneys, and the heart. However, given the limitations and difficulties of current therapies and technologies for achieving good glucose control, even most participants in the EDIC study cannot achieve the levels of control associated with reduced complications. Thus, other approaches are needed to prevent and delay progression of complications. New insights into the underlying molecular mechanisms of diabetes complications are imperative in order to develop new therapeutic approaches.

Goal VI: Attract New Talent and Apply New Technologies to Research on Type 1 Diabetes

Research on type 1 diabetes spans a broad range of scientific disciplines, including endocrinology and metabolism; immunology; genetics; epidemiology; clinical trials; neuroscience; behavioral science; cell, developmental, and vascular biology; and the physiology of the heart, eyes, kidneys, and urologic tract, and the central and peripheral nervous systems. Propelling research progress on the understanding, prevention, and cure of type 1 diabetes requires a cadre of scientists with

1998	2000	2000	2002	2002	2003
 First year of *Special Statutory Funding Program for Type 1 Diabetes Research.*	"Edmonton" Protocol improved success rate of islet transplantation from less than 5 percent to 90 percent.	Long-term follow-up of patients from DCCT suggested that the benefits of tight glucose control have a sustained effect on diabetes complications, a phenomenon called "metabolic memory."	The Diabetes Prevention Trial-Type 1 (DPT-1) demonstrated the feasibility of accurate assessment of risk for type 1 diabetes.	Research demonstrated that treating new onset type 1 diabetes patients with a monoclonal antibody preserves residual beta cell function.	The rate of kidney failure has stabilized in part due to improved management of diabetes.

diverse research training and expertise. Furthermore, it is critical for basic scientists and clinical researchers to work together to translate research findings from the bench to the bedside, and from the bedside to clinical practice, in order to achieve real improvements in patients' health and quality of life.

Powerful new technologies that have emerged over the past few years make this an exciting time to be involved in scientific research and have quickened the pace of discovery. Application of these new and emerging technologies to type 1 diabetes research provides unprecedented opportunities to solve key problems. For example, "proteomics" involves the use of novel, integrated technologies to identify and quantitate proteins and study their interactions. Identifying how protein expression changes over the course of type 1 diabetes onset and progression can help researchers understand the underlying disease processes, develop biomarkers of disease onset and progression, and propose and test novel prevention and treatment strategies. Type 1 diabetes research stands to benefit greatly from the application of proteomics and many other new and emerging technologies.

NIH TYPE 1 DIABETES RESEARCH PORTFOLIO

The NIH vigorously pursues and supports research on the understanding, prevention, and cure of type 1 diabetes. Current efforts span diverse areas, such as genetics, genomics, proteomics, immunology, developmental biology, imaging, bioengineering, glucose sensing, and insulin delivery. NIH-supported clinical trials are testing promising agents for type 1 diabetes and its complications. Type 1 diabetes research at the NIH is largely supported by regularly appropriated funds that the Department of Health and Human Services (HHS) receives for diabetes research through the Labor-HHS-Education appropriations subcommittees. In addition, it is supported by the *Special Statutory Funding Program for Type 1 Diabetes Research*, which the NIDDK administers on behalf of the Secretary and in collaboration with multiple NIH Institutes and Centers, as well as the CDC.

Critical to the national effort to combat type 1 diabetes are studies funded through investigator-initiated research grants (primarily R01 grants). The NIH vigorously supports investigator-initiated research projects, and also fosters development of research efforts in areas of particular importance and opportunity through solicitations for grant applications and research contract proposals. This type of research has provided remarkable insights about type 1 diabetes and has laid the foundation for the development of improved treatment approaches and possible prevention strategies. Much of the positive impact of NIH-supported research comes from creative, hypothesis-driven endeavors undertaken by outstanding investigators working in laboratories across the country, funded through a peer-reviewed, highly competitive process. In addition, type 1 diabetes research has benefited from the results of other major cross-cutting NIH efforts such as the wealth of genetic information flowing from the Human Genome Project and the expanded knowledge base NIH research has fueled regarding developmental and cell biology, autoimmunity, and transplantation biology.

Complementing and extending this research base, the *Special Funding Program* has furthered the creation of unique, collaborative, and innovative research consortia and clinical trials networks to increase understanding about the prevention, treatment, and cure of type 1 diabetes. Initiatives supported by this program differ in size, scope, duration, and nature from other type 1 diabetes efforts supported through regular NIH appropriations. The *Special Funding Program* enabled the initiation of most of these large-scale, high-impact efforts, at a scientifically optimal scale of operation. Importantly, the research efforts that have been supported to date have

2005

DAISY study found that genetically vulnerable newborns can be identified and followed prospectively to prevent diabetic ketoacidosis, the leading cause of diabetes-related morbidity and mortality in infants.

2005

Long-term follow-up of patients from the DCCT demonstrated that intensive therapy reduces cardiovascular complications.

2006

FDA approved the first inhaled version of insulin.

2006

First generation of FDA-approved continuous glucose monitors paired with insulin pumps pave the way for developing an artificial pancreas and closing the feedback loop between glucose levels and insulin delivery.

spurred numerous future opportunities that could dramatically improve the lives of patients with type 1 diabetes. The following are highlights of some of the major collaborative research efforts and innovative approaches that are supported by the *Special Funding Program*. These research efforts are illustrative examples and not a comprehensive overview of the entire NIH type 1 diabetes research portfolio.

Goal I: Identify the Genetic and Environmental Causes of Type 1 Diabetes

***Search for Diabetes in Youth (SEARCH)*:** There are no comprehensive population-based estimates of diabetes burden among American youth. SEARCH will define the prevalence and incidence of diabetes in children and youth less than 20 years of age in six geographically dispersed populations that encompass the ethnic diversity of the United States. This study will help increase understanding of how type 1 diabetes strikes and unfolds.

***The Environmental Determinants of Diabetes in the Young (TEDDY)*:** The goal of TEDDY is to identify environmental causes of type 1 diabetes in genetically susceptible individuals. This long-term study is enrolling at-risk newborns and then following them until they are 15 years of age. The study is crucial to helping researchers understand the environmental triggers that play a role in type 1 diabetes disease onset and development.

***Type 1 Diabetes Genetics Consortium (T1DGC)*:** T1DGC is organizing international efforts to identify genes that determine an individual's risk of developing type 1 diabetes. This Consortium is currently recruiting 2,800 families who have two or more siblings with type 1 diabetes in order to identify genes that increase susceptibility. Finding these genes will not only increase understanding of the underlying molecular mechanisms of disease development, but also aid in the discovery of novel prevention strategies and identification of patients who could benefit from these approaches.

Goal II: Prevent or Reverse Type 1 Diabetes

***Cooperative Study Group for Autoimmune Disease Prevention (Prevention Centers)*:** The mission of the Prevention Centers is to engage in scientific discovery that significantly advances knowledge about the prevention and regulation of autoimmune diseases, including type 1 diabetes. Pre-clinical research conducted by the Prevention Centers is key to the development of strategies for modulating the immune system so that they can be tested in human clinical trials.

***Immune Tolerance Network (ITN)*:** Immune tolerance is the process by which the immune system accepts a protein or other molecule as "self" and does not attempt to destroy cells or tissues containing that protein. Tolerance induction can block the autoimmune process underlying type 1 diabetes or enable the body to accept transplanted islets without the need to globally suppress the immune system. Research conducted through the ITN is evaluating new treatments to induce tolerance in type 1 diabetes, as well as other disease areas. The ITN is currently conducting and developing several clinical trials related to type 1 diabetes and islet transplantation. Research on tolerance is critical both for developing therapies to slow or reverse type 1 diabetes, as well as for improved approaches to islet transplantation.

***Standardization Programs*:** Standardized assessment of key measures for type 1 diabetes research is extremely important to ensure consistency across laboratories and clinical trial networks, so that data can be compared and combined. Efforts are ongoing to improve and standardize the measurement of autoantibodies (used to identify initiation of autoimmunity), C-peptide (a measure of beta cell mass and function), and HbA1c (a measure of long-term blood glucose control).

***Trial To Reduce IDDM in the Genetically At Risk (TRIGR)*:** This multicenter, international study is comparing the development of type 1 diabetes in genetically susceptible infants who are weaned onto a hydrolysate of cow's milk formula, in which many of the cow proteins have been broken down, versus standard cow's milk formula. TRIGR, which is currently in the patient recruitment phase, could have a major impact on disease prevention if differences are observed between the two types of formulas.

***Type 1 Diabetes TrialNet (TrialNet)*:** TrialNet is an international network of investigators, clinical centers, and core support facilities. It supports the development and implementation of clinical trials of agents to slow the progression of type 1 diabetes in new onset patients and to prevent the disease in at-risk patients. TrialNet has launched several studies that are recruiting patients and is currently evaluating several other therapeutic agents to test in the network. This type of collaborative network infrastructure is

critical for facilitating clinical trials in type 1 diabetes, as well as for making real improvements in patients' health by identifying new therapeutic agents.

Goal III: Develop Cell Replacement Therapy

***Beta Cell Biology Consortium (BCBC)*:** The mission of this Consortium is to facilitate interdisciplinary approaches that will advance understanding of pancreatic islet cell development and function. The long-term scientific goal is to develop a cell-based therapy to restore normal insulin production and action to diabetic patients. Working toward this goal, the BCBC has created and distributed important reagents that will serve the scientific community at large. Research pursued through the BCBC can ultimately help to overcome a major barrier to islet transplantation—the shortage of islets.

***Clinical Islet Transplantation Consortium (CIT)*:** The purpose of this Consortium is to develop and implement a program of single- and/or multicenter clinical studies, accompanied by mechanistic studies, in islet transplantation with or without accompanying kidney transplantation, for the treatment of type 1 diabetes. Research pursued through this Consortium aims to make improvements in the field of islet transplantation and to share the data and results with the broad scientific community.

***Collaborative Islet Transplant Registry (CITR)*:** The mission of the CITR is to expedite progress and promote safety in islet transplantation through the collection, analysis, and communication of comprehensive, current data on all islet transplants performed in North America. The CITR prepares an annual report with data on recipient and donor characteristics, pancreas procurement and islet processing, immunosuppressive medications, function of the donated islets, patients' lab results, and adverse events. This information will help to define the overall risks and benefits of islet transplantation as a treatment option for patients with type 1 diabetes.

***Immunobiology of Xenotransplantation Cooperative Research Program*:** This multi-institution Program is developing and evaluating pre-clinical porcine to non-human primate models of xenotransplantation (solid organ, tissue, or cell transplantation between species). The Program supports pre-clinical research to address immunological and physiological issues critical to the engraftment, survival, and function of xenografts. The long-term goal is to develop novel and efficacious strategies for broad clinical application of xenotransplantation.

***Islet Cell Resource Centers (ICRs)*:** The ICRs serve as regional centers that provide clinical grade human islets to investigators engaged in islet transplantation protocols throughout the country; optimize the procedures used to obtain such islets; and distribute human pancreatic islets to investigators for use in laboratory-based research studies. This resource provides high-quality islets for use in human islet transplantation research and allows researchers to continue to investigate islets in basic research studies.

***Non-human Primate Transplantation Tolerance Cooperative Study Group (NHPCSG)*:** This multi-institution Study Group was established to evaluate the safety and efficacy of novel therapies to induce immune tolerance in non-human primate models of kidney and islet transplantation. The Group also supports research on immune tolerance. Pre-clinical research conducted by this Group will help scientists move promising therapeutic agents from the laboratory into human clinical trials.

Goal IV: Prevent or Reduce Hypoglycemia in Type 1 Diabetes

***Diabetes Research in Children Network (DirecNet)*:** The focus of DirecNet is to investigate the use of technological advances in the management of type 1 diabetes in children and to develop a better understanding of hypoglycemia. The Network's goals include assessing the accuracy, efficacy, and effectiveness of continuous glucose monitoring in children with type 1 diabetes, and determining the extent to which exercise contributes to the risk of hypoglycemia. Until cell replacement therapy is a viable treatment option for children with type 1 diabetes, research on glucose sensing and insulin delivery is crucial to improving quality of life and decreasing the number of hypoglycemic episodes.

Goal V: Prevent or Reduce the Complications of Type 1 Diabetes

***Animal Models of Diabetic Complications Consortium (AMDCC)*:** The AMDCC is an interdisciplinary Consortium designed to develop animal models that closely mimic the human complications of diabetes for the purpose of studying disease pathogenesis, prevention, and treatment. The Consortium has already developed a number of

promising models for complications involving the heart, kidneys, and nervous system. Development of animal models is essential for pre-clinical drug development.

***Diabetic Retinopathy Clinical Research Network (DRCR.net)*:** Type 1 diabetes causes damage to the eyes and may lead to blindness. The DRCR.net conducts multicenter clinical research studies to test promising therapeutic agents for the treatment of two forms of diabetic eye disease—diabetic retinopathy and diabetic macular edema—and associated conditions. Because blindness is such a severe and debilitating disease complication, research pursued through this network could dramatically improve patients' quality of life.

***Epidemiology of Diabetes Interventions and Complications Study (EDIC)*:** The aim of EDIC is to study the clinical course and risk factors associated with the long-term complications of type 1 diabetes, using the cohort of the DCCT. The DCCT/EDIC research group has observed dramatic long-term benefits of intensive glucose control in preventing and delaying complications of the eyes, kidneys, nerves, and heart. These results have had a major impact on the clinical care of diabetes patients.

***Family Investigation of Nephropathy and Diabetes (FIND)*:** The FIND Consortium is carrying out studies to elucidate the genetic susceptibility to kidney disease in patients with diabetes, as well as genetic susceptibility to retinopathy in diabetic patients. A family-based study recruited more than 2,500 affected and discordant pairs of siblings. A separate case control study is completing recruitment of more than 3,000 individuals. These studies will help researchers understand the genetic underpinnings of the kidney and eye complications of diabetes, which can, in turn, inform prevention and treatment strategies.

***Genetics of Kidneys in Diabetes Study (GoKinD)*:** GoKinD was established to study the genetics of kidney disease in patients with type 1 diabetes. The study group has collected and is distributing DNA and other biological samples from more than 1,700 adults with type 1 diabetes in the United States and Canada. Scientists will use these samples to identify genes that are important in the development of, or resistance to, diabetic kidney disease.

Goal VI: Attract New Talent and Apply New Technologies to Research on Type 1 Diabetes

***Research Training and Career Development in Pediatric Diabetes*:** This program provides support of research training and career development in pediatric diabetes at institutions that have environments, mentors, and programs that will make them particularly effective in enhancing the number of independent investigators contributing to research in pediatric diabetes. The awards, through the T32 (institutional research training) and K12 (clinical scientist career development program) grant mechanisms of the NIH, are intended to provide an opportunity for continuous training from the clinical fellowship years to emergence as a fully trained independent investigator. These integrated programs are designed to prepare pediatricians, selected by the institution, for careers in pediatric endocrinology research related to diabetes.

***Type 1 Diabetes—Rapid Access to Intervention Development (T1D-RAID)*:** The T1D-RAID program provides resources for manufacture and pre-clinical development of drugs, natural products, and biologics that will be tested in type 1 diabetes clinical trials. The goal of T1D-RAID is to facilitate the translation of promising therapeutic agents from the bench to the bedside, in order to more rapidly impact patients' health.

> For more information on these and other type 1 diabetes research efforts, please visit a Web site dedicated to research supported by the *Special Statutory Funding Program for Type 1 Diabetes Research*: www.T1Diabetes.nih.gov.

Collaborative Planning Process

The NIDDK is the lead Institute at the NIH for pursuing type 1 diabetes research. Because this research involves diverse scientific disciplines, the NIDDK collaborates extensively with other NIH Institutes and Centers, as well as other government agencies. Type 1 diabetes research involves nearly every Institute and Center of the NIH, including the National Center for Research Resources (NCRR), National Eye Institute (NEI), National Human Genome Research Institute

(NHGRI), National Heart, Lung, and Blood Institute (NHLBI), National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institute of Child Health and Human Development (NICHD), National Institute of Dental and Craniofacial Research (NIDCR), National Institute of Environmental Health Sciences (NIEHS), National Institute of Neurological Disorders and Stroke (NINDS), National Institute of Nursing Research (NINR), National Library of Medicine (NLM), and other NIH Institutes and Centers that are represented on the statutory Diabetes Mellitus Interagency Coordinating Committee (DMICC). The NIH also works closely with the CDC, the FDA, the Centers for Medicare & Medicaid Services (CMS), and other governmental agencies represented on the DMICC. Also contributing to program planning are the two major diabetes voluntary organizations, the Juvenile Diabetes Research Foundation International (JDRF) and the American Diabetes Association (ADA).

DEVELOPMENT OF THE STRATEGIC PLAN

Origin and Purpose of the Plan

In January 2005, the NIDDK convened an *ad hoc* planning and evaluation meeting of external scientific and lay experts in type 1 diabetes. The purpose of the meeting was to perform a mid-course assessment of many currently funded type 1 diabetes research programs, and to identify future research opportunities within this context. The meeting focused on research consortia and clinical trials networks supported by the *Special Statutory Funding Program for Type 1 Diabetes Research*. A detailed summary of the meeting can be accessed on the NIDDK Web site at: www.niddk.nih.gov/federal/planning/Jan-18-19-T1D-FINAL.pdf.

One of the recommendations emanating from this meeting was to initiate a much broader review of the entire state-of-the-science with respect to type 1 diabetes, including recent research advances and emerging opportunities. Such a review would be far more encompassing and future-oriented than the assessment performed at the January 2005 meeting, which was largely focused on existing programs. In response to this recommendation, the NIDDK Director announced in March 2005, that the Institute would spearhead a new strategic planning effort in type 1 diabetes research under the auspices of the statutory DMICC, chaired by the NIDDK. The membership of this Committee includes all NIH components involved in diabetes research, as well as other relevant Federal agencies.

The purpose of this Strategic Plan is to serve as a scientific guidepost to the NIH and to the investigative and lay communities by identifying compelling research opportunities. These scientific opportunities will inform the priority-setting process for the type 1 diabetes research field and propel research progress on the understanding, prevention, treatment, and cure of type 1 diabetes and its complications.

Collaborative Planning Process

The Strategic Plan was developed with broad input from a diverse and talented group of researchers and lay experts dedicated to advancing type 1 diabetes research (please see Appendix A). Participants included representatives from the NIH and other Federal agencies represented on the DMICC, scientists external to the NIH, lay people representing patients' interests, and representatives from diabetes voluntary organizations.

The Strategic Plan was organized around the six overarching goals of type 1 diabetes research. To formulate the Plan, Working Groups were convened to address each of the first five goals. The sixth goal, "Attract New Talent and Apply New Technologies to Research on Type 1 Diabetes," is an overarching goal that is relevant to all of type 1 diabetes research. Therefore, this goal was addressed by each of the five Working Groups. Each Working Group was chaired by a scientist external to the NIH and was composed of other external scientific experts, a lay representative, a representative of a diabetes voluntary organization, at least one member of the DMICC, and other senior scientific Government representatives. The Working Group members were asked to survey the state-of-the-science and develop a summary of progress and opportunities relevant to each goal.

In addition to the Working Groups, the Strategic Plan was informed by insights provided by an overarching Executive Committee, composed of the chairs of the five Working Groups and representatives from the government and from

diabetes voluntary organizations. The Executive Committee met on September 28, 2005, to ensure that in aggregate the components developed by the Working Groups were comprehensive and addressed the most compelling opportunities for the prevention, therapy, and cure of type 1 diabetes and its complications. The Executive Committee provided guidance on integrating the products of each Working Group into a final Strategic Plan that will serve the purpose of informing future priority-setting in type 1 diabetes research.

To solicit broad public input into the strategic planning process, a draft document was posted on the Strategic Plan's Web site (accessed at: www.T1Diabetes.nih.gov/plan) for a month-long period of public comment. Scientists with expertise relevant to type 1 diabetes and its complications and members of voluntary and professional health advocacy organizations were invited to comment. A broad range of expertise was represented among the individuals and organizations providing vigorous input on the draft Strategic Plan.

Organization of the Strategic Plan

Based on the same general content, two versions of this Plan have been developed for: (1) patients with type 1 diabetes, their family members, and the public, and (2) the scientific research community. Both versions contain a summary of major research objectives.

The version of the Plan for patients and the public describes how achieving the goals will directly benefit the health and quality of life of patients with type 1 diabetes and their family members. Each Goal includes the following sections:

- *Why the Goal Is Important:* This section highlights the clinical relevance of the goal and describes how research progress can have a direct and dramatic impact on the lives of patients with type 1 diabetes and their family members.
- *Profiles of Patients or Scientists:* This section describes the impact of type 1 diabetes on the lives of patients with type 1 diabetes and family members, or the experiences of researchers studying the disease.

The technical version of the Plan provides greater detail regarding specific research directions that can be pursued to achieve the overarching goals of type 1 diabetes research. Under each Goal, the following sections are included:

- *Introduction and Background:* A brief description of the current state-of-the-science, and an overview of the importance of the goal in propelling research progress in type 1 diabetes.
- *Recent Scientific Advances:* Examples of major achievements in type 1 diabetes research that have made a significant impact on the research field or patients' health, particularly in the last 5 to 7 years.
- *Research Objectives and Strategies To Achieve Goals:* The objectives are specific research directions that can be pursued to realize the goal of the chapter. The objectives were identified by Working Group members as being critically important for overcoming current barriers and achieving progress in type 1 diabetes research relative to the chapter's overarching goal over the next 10 years. This section also describes some immediate steps that can be taken to achieve the research objectives.

Implementation of the Strategic Plan

Successful implementation of the research objectives outlined in this Strategic Plan requires the collaboration of the multiple Institutes and Centers of the NIH, other government agencies represented on the DMICC, industry, and the diabetes research and voluntary communities. It is only through the involvement and collaboration of these different entities that research progress will be realized.

Although this document, which reflects current research advances and objectives, is necessarily "static," the strategic planning process is dynamic. Novel findings and new technologies can dramatically and positively change the course of planned research. Therefore, to be successful, this Strategic Plan must be periodically assessed by scientific experts in the type 1 diabetes research field, so that new and emerging opportunities can be identified. The DMICC will continue to serve an important role by assessing progress toward attaining the goals and objectives described in this Strategic Plan. The NIH will also continue to solicit input from the external scientific community through forums such as scientific workshops, conferences, and planning and evaluation meetings. This input will continue to be a valuable and necessary component of the NIH's strategic planning process for type 1 diabetes research.

LOOKING FORWARD: FUTURE TYPE 1 DIABETES RESEARCH

Research efforts over the past several decades have led to tremendous improvements in the health and quality of life of patients with type 1 diabetes. The prognosis for newly diagnosed patients has dramatically improved compared to just a decade ago. While these improvements are positive, one thing remains certain: we are not there yet. People with type 1 diabetes still check their blood glucose levels, administer insulin, and develop life-threatening complications. It is imperative to build upon the strong existing research base to not only improve current treatment strategies, but also identify ways to prevent and cure the disease. Because of new and emerging technologies in areas such as genomics, imaging, and systems biology, there is great potential to make significant and dramatic improvements in the health of patients with type 1 diabetes in the near future. Thus, it is important to harness these technologies for type 1 diabetes research and to sustain and intensify the momentum that currently exists in the field. Achieving the specific objectives and making progress toward the overarching research goals outlined in this Strategic Plan will have an enormous impact on patients with type 1 diabetes, as well as on patients with other forms of diabetes and other autoimmune diseases.

Figure Legend: Type 1 diabetes results from an interplay of genetic and environmental factors. A newborn (teal shading) with either a parent or sibling (blue shading) with type 1 diabetes has a greater chance of developing the disease than does a child with no family history. The Environmental Determinants of Diabetes in the Young (TEDDY) study will be following genetically at-risk infants through adolescence to try to identify environmental factors that may trigger disease onset. *(Image modified from figure courtesy of Dr. Marian Rewers and the Diabetes Autoimmunity Study in the Young [DAISY].)*

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.

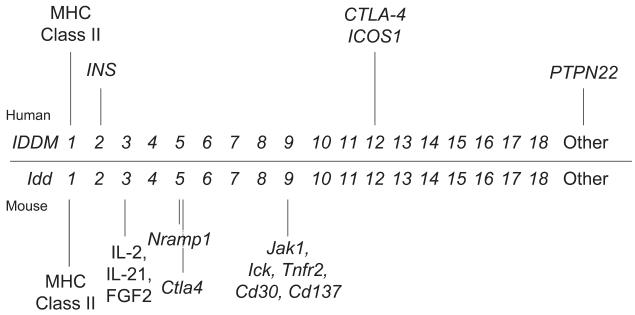
Contribution of *INS* to Type 1 Diabetes Susceptibility: A series of studies has confirmed an association of type 1 diabetes with the insulin gene, *INS*, and in particular, that susceptibility to type 1 diabetes is likely to be directly influenced by the number of repeated elements in the *INS* gene, 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 multidisciplinary 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 intrauterine 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.

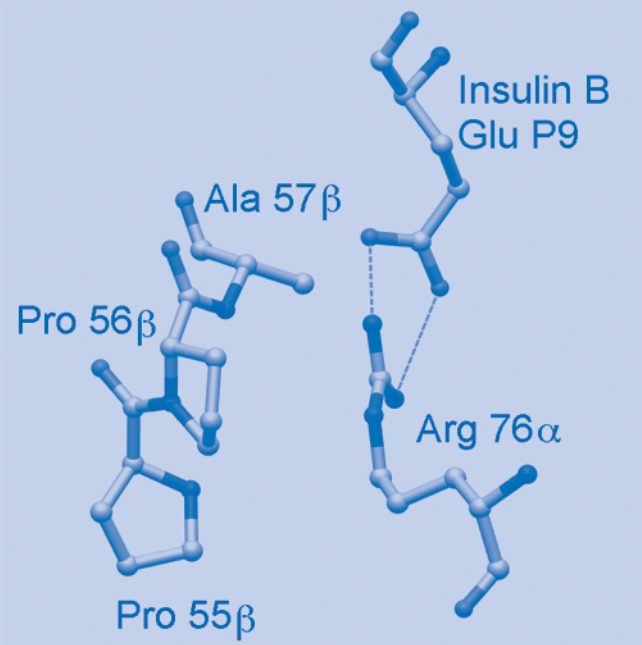
- *Explore candidate environmental agents (e.g., food elements, toxins, stress, infectious agents) as triggers for islet autoimmunity and type 1 diabetes in animal models of type 1 diabetes.*

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.

Figure Legend: Three-dimensional representation of key amino acid interactions between insulin and a major histocompatibility complex (MHC) Class II molecule, DQ8, associated with a predisposition for type 1 diabetes. *(Image courtesy of Dr. Kai Wucherpfennig, Dana-Farber Cancer Institute and Harvard Medical School.)*



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.

RECENT SCIENTIFIC ADVANCES

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 dystrophia myotonica 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 difficult 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 autoantibodies, 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).*

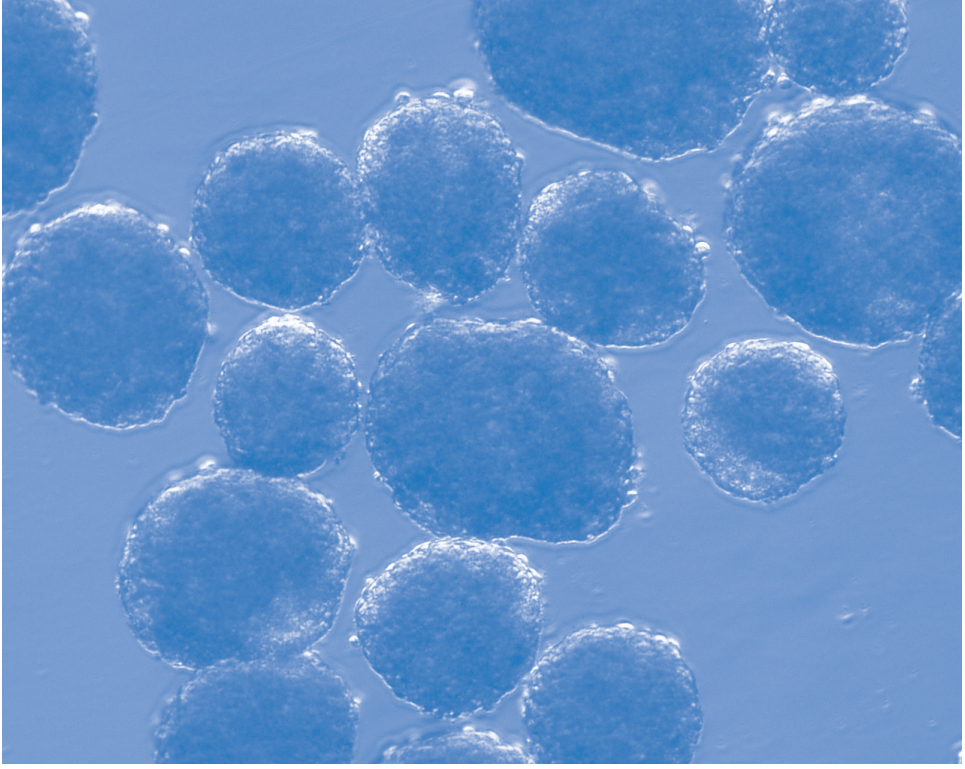
The immature intestine allows leakage of undigested dietary proteins that may be antigenic. Although the causes of diabetic autoimmunity in humans remain controversial, studies in diabetes-prone mice and rats show that hypoantigenic weaning diets are protective. TRIGR seeks to determine whether the risk 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 reposited 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.

Figure Legend: Human islets. *(Image courtesy of Dr. Camillo Ricordi and Mr. Over Cabrera, University of Miami, Diabetes Research Institute.)*



GOAL III:

DEVELOP CELL REPLACEMENT THERAPY

Recent Scientific Advances

Islet Transplantation: A Viable Therapeutic Alternative for Some Patients

- Infrastructure Creation To Improve Islet Isolation and Promote Basic Islet Research
- Achievement of Insulin Independence Using Islets from a Single Donor and Partial Pancreas
- Induction of Immune Tolerance and Development of Therapeutic Agent Pipeline
- Immune Monitoring for Early Diagnosis of Rejection and Tolerance

Islet Cell Biology

- New Technology To Study Developmental Biology of the Endocrine Pancreas
- Role of Master Control Genes in Regulating Formation of Pancreatic Beta Cells
- Recognition of the Regeneration Potential of Pancreatic Beta Cells
- Steps Toward the Creation of New Beta Cells from Stem Cells
- Imaging the Pancreatic Islet

Research Objectives and Strategies To Achieve Goals

Islet Transplantation

- Develop Novel Strategies and Infrastructure That Support Advancing Pancreas Procurement and Islet Processing
- Develop Improved Methods To Assess Islet Beta Cell Viability and Function That Predict Early Islet Function After Transplant
- Investigate the Use of Porcine Islets as an Alternate Source of Islets for Transplantation
- Improve Islet Transplant Procedures
- Develop Novel Methods To Accurately Assess the Post-Transplant Islet Mass
- Harness New Understanding of the Immune System To Develop Improved Clinical Monitoring and Immunotherapies

Pancreatic Development, Stem Cells, and Regeneration

- Grow a Renewable Supply of Pancreatic Beta Cells That Can Be Transplanted into Patients
- Understand How Mature Beta Cells Are Maintained and Replenished in the Adult Pancreas
- Develop Strategies To Regenerate Beta Cells Through Replication or Neogenesis

STRATEGIES TO DEVELOP CELL REPLACEMENT THERAPY

INTRODUCTION AND BACKGROUND

Islet transplantation has shown promise as a treatment strategy for type 1 diabetes, particularly in patients who suffer from recurrent bouts of hypoglycemia or who have already undergone a kidney transplant. Although the improvements in success rates with the therapy have brought tremendous hope for a cure, formidable obstacles impede widespread implementation of islet transplantation.

First, there is an inadequate supply of donor pancreata for the number of potential recipients. Researchers are seeking ways to optimize both the organ procurement and the islet isolation processes from these precious and finite resources. Consonant with these efforts, research is in progress to determine whether progenitor/stem cells[2] or genetically modified pancreatic cells could be "coaxed" to develop into islets or beta cells, and thus provide an unlimited source of cells for transplantation. Ongoing research in regeneration is also determining if adult beta cells could be coaxed to form more beta cells (replication) or if other resident cell types could be directed toward a beta cell fate (neogenesis). Researchers are also investigating the potential use of porcine islets as an alternative to human islets.

Following transplantation, substantial islet cell death and dysfunction occur within the first few hours and days. Unlike whole organ transplantation, in which blood flow to the transplanted organ is immediately restored, islets do not have an intact blood vessel system (vasculature). A rapid new growth of blood vessels is an absolute requirement for islet survival and function. Furthermore, early inflammation at the site of transplantation, and even the diabetic environment itself, could contribute to rapid programmed cell death (apoptosis). It is important for researchers to identify ways to promote successful islet engraftment and survival so that patients require fewer islets and/or transplants to produce sufficient amounts of insulin.

Another major obstacle to islet transplantation is the requirement for lifelong immunosuppression. Drug intervention is required to prevent rejection of transplanted islets and to prevent recurrence of the underlying autoimmunity that initiated the disease. However, these drugs can cause serious and adverse side effects. Research is crucial to identify less toxic methods to prevent islet rejection and the recurrence of autoimmunity, in order to realize the promise of islet transplantation. Currently, only adult patients with exceptionally brittle diabetes and recurrent hypoglycemia, or patients with end-stage renal disease, are eligible for this experimental therapy due to the toxicity associated with the required immunosuppressive medicines.

New technologies on the horizon may dramatically improve the success of islet transplantation. For example, new *in vivo* imaging technologies are being developed that would allow transplant surgeons to "see" the islets after transplantation and monitor the effectiveness of transplantation therapy in patients. The translation of this technology from animal models to human patients would constitute a real breakthrough in the ability to detect disease and to monitor treatment in patients with type 1 diabetes.

Cell replacement therapy can eliminate not only the need for the endless finger sticks and needles, as well as anxiety, currently endured each day by type 1 diabetes patients, but also the devastating long-term disease complications. If cell replacement therapy could be done with reduced toxicity, many more people besides the limited group with exceptionally severe hypoglycemia or renal failure could benefit.
To make the widespread use of this therapy a reality, it is imperative to promote basic research in islet cell biology—to understand beta cell development and regeneration—as well as clinical research in islet transplantation. From both basic and clinical avenues of research will emerge strategies to permit long-term post-transplant function of the insulin-producing cells without the need for lifelong recipient immunosuppression.

[2] The NIH supports human embryonic stem cell research consistent with federal funding policies.

Islet Transplantation: A Viable Therapeutic Alternative for Some Patients

Within the past 5 years, transplantation of human pancreatic islets has been shown to reproducibly reverse debilitating severe hypoglycemic episodes and enable some patients to become insulin independent. These impressive findings, first reported by the Edmonton group, have led to a renewed interest in the islet transplantation field. The dissemination of the successful Edmonton Protocol from the original few centers to sites across the United States and throughout the world was an important research achievement. The Edmonton findings have now been confirmed by independent centers throughout North America and Europe. An international, multicenter trial of the Edmonton Protocol was completed by the Immune Tolerance Network at nine sites, showing that the protocol could be replicated with success, depending on the experience of the site. Worldwide, more than 500 patients have been treated since 2000 (19). The data obtained by the centers in North America are available through the Collaborative Islet Transplant Registry (CITR), which was established and funded by NIH to compile and analyze this information. Analysis of the data clearly shows that islet transplantation is a viable therapeutic alternative for some patients. The ability to successfully disseminate the Edmonton Protocol has also spurred the creation of a recently launched NIH-supported Clinical Islet Transplantation Consortium, which is building upon the success of the Edmonton Protocol to further improve the safety and long-term success of methods for islet transplantation. The number of transplant centers performing clinical islet transplantation continues to increase, and insulin independence without hypoglycemic episodes is now an achievable goal for some patients.

Infrastructure Creation To Improve Islet Isolation and Promote Basic Islet Research: Pilot clinical trials have demonstrated that insulin independence and long-term islet graft function could be obtained not only with islets processed and transplanted at the same institution, but also with islets processed at regional NIH-funded Islet Cell Resource Centers (ICRs) and shipped for transplantation at remote institutions across the United States. This success has validated the concept that regional centers could be utilized for islet cell processing and distribution. Furthermore, the establishment of the ICRs has enabled an infrastructure that permits collaborative optimization of pancreas shipping devices, preservation media, islet isolation technology, and interim storage through comparative assessments. The collaboration has also led to the identification of salient roadblocks to large-scale islet production and transplantation. The ICRs provide resources, structure, and a coordinated community of investigators focused on enhancing the quality of isolated islets, promoting basic islet research, and enabling additional facilities to perform the procedures. The ICRs work closely with the CITR to collate and disseminate data on islet procurement and production, as well as on clinical outcomes following transplantation in North America. This joint effort facilitates comparative analyses that will eventually define the safest and most effective clinical protocols.

Achievement of Insulin Independence Using Islets from a Single Donor and Partial Pancreas: The improved success rates of islet transplantation have largely been achieved using islets isolated from two or three donor pancreata. Recently, success has been realized using both single donors and islets isolated from part of a pancreas taken from a living donor. One study tested a single-donor procedure on eight patients with type 1 diabetes and found that all patients achieved insulin independence and freedom from hypoglycemia. Five patients remained insulin-independent for more than 1 year. Another study showed that islets could be isolated from a portion of the pancreas of a closely related living donor and transplanted into a patient with type 1 diabetes. Successful transplantation with fewer islets can be attributed to improved isolation procedures resulting in increased islet viability and survival. Thus, improved islet isolation procedures in the future could help to overcome the current barrier regarding the shortage of islets available for transplantation.

Induction of Immune Tolerance and Development of Therapeutic Agent Pipeline: As described in the preceding chapters, the immune system has the complex task of recognizing foreign molecules, while disregarding molecules native to the host ("tolerance"). Long-term islet graft survival in animals without the requirement for long-term immunosuppression has been demonstrated using methods that suppress costimulatory signals (key signals for discriminating between foreign and self molecules) to immune cells. The success achieved in animal models showing sustained islet graft survival and reversal of diabetes after discontinuation of all anti-rejection drugs suggests that tolerance may also be achievable in humans. Monoclonal antibodies, other blocking agents, and selected cytokine combinations are under intense investigation to prevent immune activation and induce

immune tolerance in islet transplantation. Identification of novel therapeutic agents promises to overcome the current barrier imposed by the requirement for chronic immunosuppressive therapy after transplantation. These same therapeutic agents may also have added utility for preventing or reversing the initial autoimmune process. Some new agents studied in other diseases and/or animal models are already moving into pilot clinical trials of islet transplantation. Additional novel molecules and drugs are currently in the pipeline, or are already at the pre-clinical level of testing. These molecules may soon become available for pilot clinical trials of islet transplantation. These new therapeutic agents may enable more effective modulation of the immune response, while also decreasing the side effects associated with chronic administration of currently available anti-rejection drugs.

Immune Monitoring for Early Diagnosis of Rejection and Tolerance: Many clinical and biological markers can be used to determine if a solid organ graft is being rejected. In contrast, there is no biochemical marker for islet rejection that enables detection of islet loss early enough following transplantation to permit effective intervention and rescue. At the time of documented hyperglycemia and need for return to exogenous insulin administration, significant islet loss has already occurred. This observation is similar to the situation that occurs at the onset of type 1 diabetes, as described in the previous chapters. Scientists have recently demonstrated elevated expression of several key genes in the peripheral blood associated with inflammation—an event that precedes clinical evidence of post-transplant islet loss. Gene expression profiles may serve as molecular signatures that foretell impending graft rejection. In addition, these profiles may provide predictive guideposts for withdrawal of immunosuppression. Early detection of destructive processes will guide the development of effective intervention strategies to reverse immune activation after islet transplantation, before islet cell destruction occurs.

Islet Cell Biology

Many scientific advances over the past 5 years have markedly advanced the understanding of how pancreatic beta cells develop and function, as well as how they are adversely affected in type 1 diabetes. These advances have been due largely to new information flowing from completion of the human and mouse genome sequencing projects and the development of novel technologies that have enabled researchers to study the genes expressed in the pancreas—both in humans and in a variety of model organisms. Research progress in this field has also been accelerated by the creation of the Beta Cell Biology Consortium (BCBC), which consists of a team of researchers who generate publicly available resources and tools that could greatly advance the study of beta cell development and regeneration.

New Technology To Study Developmental Biology of the Endocrine Pancreas: A few key genes code for special transcription factor proteins that regulate the expression of many other genes. To understand the necessary steps for a progenitor/stem cell to develop into a beta cell, it is important to identify the transcription factors and the downstream target genes that mediate this transition. A major barrier to determining these steps is that these progenitor cells are transient in nature and are found in vanishingly small numbers. Furthermore, no methods exist to prospectively identify and isolate purified populations of pancreatic progenitor/stem cell populations. Recently, scientists in the BCBC have created mouse models that allow researchers to visually track the expression of transcription factors that characterize pancreatic progenitors at various stages of progression toward mature beta cells. Using these genetically engineered mice, researchers could isolate pancreatic beta cells using an experimental technique called fluorescence activated cell sorting (FACS). This advanced technology yields pure populations of mouse pancreatic beta cells at different stages of development. These cell populations can then be used to gain further insights into which genes regulate beta cell development and function. This approach will enable researchers to identify appropriate cell surface markers on pancreatic progenitor cells. Pursuit of this research avenue could pave the way to the isolation and prospective purification of human progenitor cell populations that will mature into insulin-producing beta cells.

Role of Master Control Genes in Regulating Formation of Pancreatic Beta Cells: Researchers have identified important transcription factors that have essential roles in either the formation or the function of the pancreas, pancreatic islets, or pancreatic beta cells. When mutated, some of the transcriptional regulators expressed in pancreatic beta cells during development have been found to cause rare forms of diabetes mellitus termed Maturity Onset Diabetes of the Young (MODY). Identification of many of these transcription factors was the result of years of systematic studies of the insulin promoter, the part of the insulin gene that regulates its expression. This research pinpointed specific regulatory DNA sequences within the promoter and the transcription factor proteins that bind to them. Other key genes were serendipitously discovered. For instance, the gene *neurogenin 3* (*ngn 3*), which was being studied for a possible role in brain development, was found to be essential for the formation of

pancreatic islets, including beta cells. The identification and molecular characterization of key transcription factors, such as *Pdx-1* (a master regulator for formation of the endocrine pancreas and a MODY gene), provide a starting point for understanding the complex gene regulatory networks that exist within both the pancreatic progenitor cells and the mature beta cells. These studies can help researchers identify the necessary steps to turn progenitor/stem cells into insulin-producing beta cells.

Recognition of the Regeneration Potential of Pancreatic Beta Cells: Evidence gained over the past 5 years indicates that both humans and animals have some ability to regenerate beta cells. Thus, it may be possible to restore beta cell mass in type 1 diabetes patients whose beta cells are not completely destroyed. Many tissues have been found to contain progenitor/stem cells that could restore lost cell types. However, it is not yet clear whether such a cell type exists in the pancreas or, if so, whether it can form new beta cells after all existing ones have been damaged or destroyed. Recent studies in mice imply that the proliferation of new beta cells after injury contributes predominantly to the new beta cell population. This observation contradicts older models of pancreas regeneration. These novel findings need to be expanded, in order to enhance understanding of the regenerative potential of beta cells and other resident pancreatic cells, as well as to determine whether regeneration is a clinically significant process. A dampening of autoimmunity through intervention with Freund's complete adjuvant has been reported to reverse autoimmune diabetes in mice. The reversal was attributed to a restoration of beta cell mass that occurred through regeneration and suggests that it may be possible to devise similar regeneration approaches in humans. In humans, there is morphological evidence for beta cell regeneration, even in patients with long-standing type 1 diabetes. Such studies are encouraging because, even if the number of residual beta cells is small, perhaps only a few cells may be needed to generate sufficient numbers of cells to restore lost beta cell function in a patient with type 1 diabetes.

Steps Toward the Creation of New Beta Cells from Stem Cells: In 1998, researchers reported deriving the very first human embryonic stem (ES) cells. Since then, the potential of inducing human ES cells to form a wide variety of other cell types has been demonstrated by several groups of researchers. With these remarkable advances, the possibility has emerged of generating from human ES cells large quantities of either pancreatic beta cells or whole pancreatic islets. However, several barriers have become apparent as a result of attempts to convert human ES cells into insulin-secreting beta cells. The major barrier is the lack of knowledge about how to direct the differentiation of ES cells, or any other progenitor/stem cell type, toward a pancreatic beta cell fate. It has become clear that ES cells, or any other starting cell type, may need to be induced to pass through many intermediate cell fates, just as occurs when pancreatic beta cells are formed during development. This avenue of research has the potential to create an unlimited supply of islets for transplantation, which can help to overcome the current clinical barrier created by an insufficient number of available cadaveric donor pancreata.

Imaging the Pancreatic Islet: Since 1999, there has been significant progress toward directly visualizing the pancreatic beta cells, transplanted islets, and inflammation of type 1 diabetes using imaging technologies, particularly positron emission tomography (PET) and magnetic resonance imaging (MRI) (see Goal VI). Isolated human islets have been labeled with nontoxic imaging agents that allow them to be seen after transplantation into animals. Targeting molecules are being developed that can carry imaging agents directly to proteins on the beta cell surface, in order to count the number of beta cells in people. The visualization of early beta cell loss would enable imaging to be used as a noninvasive diagnostic tool for type 1 diabetes, as well as a means to follow the progression of the disease and monitor the effectiveness of transplantation therapy in patients. When the pancreas is under attack by the immune system, its blood vessels become "leaky." This process can be visualized through use of an imaging molecule that moves from the blood into the inflamed tissue. This ability to actually see the cells and processes associated with disease *in vivo* could help researchers better understand the life cycle of the islet and how it is damaged in diabetes.

Islet Transplantation

The overall clinical experience in islet transplantation has highlighted the complexity of a sequential approach that can last days between pancreas procurement, islet processing, pre-transplant culture, recipient immune-conditioning, and the final islet infusion. To maximize the use of scarce donor islets and minimize the risk of procedure-related complications, highly specialized and multidisciplinary teams are required. It is necessary not only to obtain uniformly high-quality islet cell products, but also to ensure optimal islet infusion techniques, infusion sites, and effective post-transplant patient management. A major barrier to transplantation is the immune response of the recipient against the donor islets. The toxicity associated with current immunosuppression makes islet transplantation appropriate for only a very limited subset of patients with type 1 diabetes. Moreover, although current protocols achieve remarkable success in achieving immediate insulin independence and/or improved glucose control without severe hypoglycemia, longer-term follow-up suggests that this success wanes over time. Chronic rejection and recurrent autoimmunity are major challenges to be overcome in this regard. The following research objectives are critically important for a stepwise, integrated approach to develop successful cell replacement strategies to treat diabetes.

Research Objective—Develop Novel Strategies and Infrastructure That Support Advancing Pancreas Procurement and Islet Processing:

- *Study potential donor interventions that minimize the negative effects of brain death and ischemia (low blood supply)/hypoxia (low oxygen) on islet survival and function.*
- *Develop improved preservation medium, shipping containers, and monitoring technologies to improve pancreas preservation during transport.*
- *Develop improved islet isolation and purification methods and novel methods for tissue processing, beyond the currently available enzyme-blend techniques.*
- *Develop new strategies to improve pre-transplant islet culture that will sustain graft survival and function.*

Prior to transplantation, islet grafts have been exposed to a series of nonphysiological conditions that sequentially contribute to a progressive reduction of the original islet cell mass. This loss of insulin-producing tissue results from the collective incremental effects of many factors, including the molecular effects of donor injuries preceding islet isolation associated with brain death and hypoxia; prolonged time of pancreas cold preservation and shipment to the islet cell processing center; enzyme-based tissue digestion techniques; and islet purification steps and pre-transplant culture/shipment to the final transplant facility. Improvement at all phases of these processes and technologies will be critically important to minimize islet loss and maximize the potential use of each donor pancreas. For example, potential strategies could include the delivery of anti-inflammatory agents and/or agents that enable survival of the pancreatic islets prior to procurement. These improvements may ultimately allow transplantation of sufficient numbers of islets obtained from only a segment of donor pancreas, such as in the case of living donor islet transplantation.

Research Objective—Develop Improved Methods To Assess Islet Beta Cell Viability and Function That Predict Early Islet Function After Transplant:

- *Define and implement novel strategies and methods for assessment of beta cell-specific viability and function.*
- *Develop predictive tests to determine the suitability of an islet cell product for clinical use (i.e., tests predictive of post-transplant survival and function).*

To predict transplant success, improved assays to assess islet quality are needed that report on beta cell-specific parameters, rather than general traits, such as oxygen consumption. Such assays are needed because the ratio of beta cells to other pancreatic cells can vary greatly among preparations, and robust pancreatic exocrine cells in a partially purified islet preparation can easily utilize enough oxygen to mask damaged beta cells. The NIH has established ICRs across the country to provide high-quality human islets for treatment of type 1 diabetes and for basic research. The ICRs share a mission to improve the quantity and quality of available human islet tissue. They have been pivotal in establishing some uniform measures of "functional" testing of islets. These measures already include testing of glucose-stimulated insulin release, but research would benefit from new surrogate markers of islet quality, which are beta cell-specific and correlate well with engraftment potential. Toward this end, research teams that include cell biologists and clinicians should work together to establish and standardize functional testing, and to identify islet factors that predict success or failure in achieving insulin independence. Candidate assays include apoptosis markers and measures of cell function, measures

of beta cell antigenicity, as well as characterization based on proteomic or genomic technologies. An adequate measure of beta cell function would correlate well with the ability of the islets to restore insulin independence in an appropriate animal model (e.g., NOD-scid mice) and in human transplantation. Once such tests are established, it will be important to identify factors in the islets that contribute to engraftment success and failure.

Research Objective—Investigate the Use of Porcine Islets as an Alternate Source of Islets for Transplantation:

- *Develop strategies to overcome hyperacute rejection.*
- *Address immunological barriers to xenotransplantation.*
- *Pursue regimens for immune tolerance induction to xenografts.*

Xenotransplantation offers a potential solution to the severe shortage of pancreata needed to treat patients with type 1 diabetes. Currently, the swine is the primary species of interest, due to its favorable reproductive capacity, as well as anatomical and physiological similarities to humans. However, xenotransplantation currently poses significant challenges, including: the immune response of the recipient against the xenograft; the physiological limitations of proper functioning of the transplanted organ/tissue; and potential transmission of infectious agents, such as porcine endogenous retrovirus, from the graft to the recipient. Recently, researchers have genetically engineered pigs whose organs are not subject to hyperacute rejection when transplanted into patients and that can be used for future research studies of xenotransplantation. The NIH is supporting a research consortium studying pre-clinical, porcine to non-human primate models of xenotransplantation. Future efforts will build upon novel preclinical findings toward a goal of making xenotransplantation a viable therapeutic strategy for patients with type 1 diabetes.

Research Objective—Improve Islet Transplant Procedures:

- *Determine the optimal sites for islet transplantation.*
- *Develop novel islet survival strategies.*

Currently, the liver is the preferred site for islet transplantation, in which islets are infused into the portal vein. However, the liver is a suboptimal or even hostile environment for transplanted islets, which may contribute to limited islet longevity. After infusion into the liver and upon entering the blood stream, islets are immediately exposed to a chemical assault. For example, islets embedded in the liver are exposed to above normal amounts of metabolic toxins and are subjected to high levels of toxic immunosuppressive drugs. Prior to engraftment, islets must also survive without the assistance of an intact network of blood vessels (in contrast with whole organ transplants). Therefore, novel approaches are needed to ensure engraftment immediately after transplant, as well as to increase the lifetime of the graft. Islets transplanted into the liver have been shown to lose their ability to respond to hypoglycemia (termed a "counter-regulatory response"). This impaired response is not seen when the islets are placed in other sites. Furthermore, changes in liver structure have been recently described following some cases of islet transplantation. Possible alternatives to the liver as a site for islet engraftment include the spleen, omentum, pancreas, and muscle. Several of these alternative sites would have the added advantage of enabling clinician scientists to retrieve and replace grafts. Preventing early post-transplant islet loss remains a challenge, but is critical to graft survival.

Research Objective—Develop Novel Methods To Accurately Assess the Post-Transplant Islet Mass:

- *Define and implement post-transplant metabolic testing of the transplant recipients to estimate: (1) functional islet mass that successfully engrafted, and (2) eventual changes in functional islet mass in long-term post-transplants.*
- *Develop novel strategies for imaging islet cells post-transplant and/or in the native pancreas (PET, MRI, video-endoscopy, in vivo microscopy).*

It is essential to develop novel methods to accurately assess the mass of insulin-producing tissue that successfully engrafts post-transplant. Both metabolic and imaging strategies are needed to define islet survival during the early post-transplant period as compared to the islet mass initially infused. These methods could permit monitoring of long-term changes in islet mass not only at the transplant site, but also in the pancreas of the patient, in order to assess, for example, the effect of therapeutic strategies on beta cell regeneration in the native pancreas. Achieving this objective will require collaboration among physicians, imaging experts, chemists, and biologists (discussed more fully under Goal VI).

Research Objective—Harness New Understanding of the Immune System To Develop Improved Clinical Monitoring and Immunotherapies:

- *Identify markers of immune rejection and recurrent autoimmunity.*
- *Define effective strategies for immunomodulation of the recipient immune response and for tolerance induction following islet transplantation.*

- *Develop effective strategies for T cell regulation.*
- *Develop novel strategies for costimulatory blockade and expansion of candidate humanized monoclonal antibodies for costimulatory blockade.*
- *Employ tissue engineering strategies to protect transplanted islets from immune cell destruction.*

There is a great need for biochemical markers that are accessible in the peripheral blood and can be used for the timely detection of islet loss; assessment of inflammation; and effective intervention and rescue, when needed. Research is warranted to assess lymphocyte gene expression in blood as a means to monitor rejection. More research is also needed to develop complementary methods for monitoring early immune activation, such as noninvasive imaging before islet cell destruction occurs. As described in Goal II, promising areas of research include the development of cellular functional assays, as well as biomarkers based on broad measurements of gene expression, including proteomics. Such assessments could help provide the scientific rationale and guidance for the optimal time to adjust and eventually discontinue immune therapy. Novel strategies of immunomodulation have recently demonstrated in animals that islet transplantation could reverse diabetes without a requirement for continuous immunosuppression. Long-term survival of mismatched islets that enabled 100 percent insulin independence with normal blood glucose levels has been achieved in diabetic non-human primates treated with a brief course of costimulatory blockade. If comparable success using these reduced levels of toxic immunosuppression could be reproduced in patients with diabetes, clinical islet transplantation may ultimately provide a curative therapy. The limited exposure to immunosuppression is key because, as previously discussed in Goal II, risks accompanying continuous immunosuppression limit the numbers of potential beneficiaries for this procedure. Therefore, extension of translational research efforts is still required to define novel immune interventional strategies aimed at blocking costimulation. Efforts must be expanded to understand relevant mechanisms of immune regulation, especially those that induce specific tolerance to the graft. Additionally, researchers should explore novel strategies, such as those that exploit T cell regulation and the infusion of donor immune and progenitor cells to achieve tolerance. Tissue engineering strategies that incorporate materials or devices that keep the islets isolated from the immune system could also be enormously helpful in shielding islets from rejection (see Goal VI). Collectively, these objectives represent overlapping aims also shared with the prevention or reversal of type 1 diabetes in its early stages.

Pancreatic Development, Stem Cells, and Regeneration

Furthering basic research in developmental and stem cell biology of the pancreas would greatly enhance efforts to produce an abundant supply of pancreatic beta cells for transplantation, or to restore to normal the mass of damaged or destroyed beta cells in individuals with type 1 diabetes. It is currently unknown which stem/progenitor cells may be the most useful as a possible source of islets for transplantation. Thus, it is important to support research using different types of stem cells, including embryonic, adult, and cord blood, as well as studies of both human and animal cells, because research on each type will build knowledge of how beta cells are formed and maintained.

Of paramount importance is the creation and free distribution of reagents of particular usefulness to beta cell biology research, such as polyclonal and monoclonal antibodies and relevant animal models and cell lines, as well as the application of tools of functional genomics and proteomics. These key reagents and resources are needed to accelerate research focused on identifying, isolating, and differentiating islet stem/progenitor cell populations to generate beta cells, as well as to propel research on understanding mechanisms of beta cell regeneration. The creation and utilization of these key resources will open new avenues toward the development of cell-based therapies in diabetes.

Research Objective—Grow a Renewable Supply of Pancreatic Beta Cells That Can Be Transplanted into Patients:

- *Identify and characterize genes that play particularly critical roles in the formation of the pancreas.*

By understanding pancreatic development, researchers may be able to recapitulate normal development of beta cells in tissue culture using stem cells obtained from the patient or from other human donors. It will be necessary to perform more standardized and highly defined studies of both the gene and protein expression profiles of pancreatic beta cells and their progenitors during development. One strategy is the application of bioinformatics tools, as they become available, for modeling the sequential activation or repression of genes during pancreatic organogenesis. These studies should be directed at identifying, defining, and then characterizing the changes that occur in gene regulatory networks. Detailed genetic studies are also needed to understand the functional

importance of these regulatory genes in a mammalian model organism such as the mouse. While the mouse should remain the primary model system, other genetic model systems such as zebrafish could be exploited for discovering novel genes and pathways involved in pancreatic development. Characterizing the regulatory mechanisms that underlie the formation of the endocrine pancreas will provide the basis for understanding how to grow pancreatic islets in the laboratory for ultimate use in islet transplantation. Another fundamental question to address is how newly forming beta cells acquire their antigenicity. Identifying and characterizing genes that play a role in this process will allow investigators to test whether type 1 diabetes is initiated by a defect in the beta cell—research that could provide new information about the etiology of type 1 diabetes.

- *Develop reagents and protocols for isolating pancreatic endocrine progenitor cells.*

The development of monoclonal antibodies to cell surface markers of beta cell precursors would allow the rapid isolation of target populations after ES cells have been induced to differentiate. To this end, key resources need to be developed, including a collection of mouse and human ES cell lines that are genetically tagged with markers that faithfully report the expression of genes specific to pancreatic progenitor cell types. Together, these research resources would enable the prospective isolation of progenitor intermediates, one of the first steps toward making pancreatic islets in culture.

- *Identify growth conditions that permit the stepwise differentiation of beta cells from stem cells or precursor cells.*

A multitude of signaling pathways is active during formation of the endocrine pancreas. However, new research is needed to determine the correct combinations of growth factor signals required at each stage of development. Furthermore, high-throughput screens are needed to identify novel, small molecules that would enhance stage-specific differentiation of the endocrine pancreas and expansion of progenitor cells. Accruing this missing knowledge would allow the creation of a protocol to both expand and differentiate cells—a key step in the development of a stem/progenitor cell-based replacement therapy.

- *Develop animal models to test the engraftment, survival, and metabolic impact of beta cells or islets derived in culture from stem/progenitor cells.*

Quantitative transplantation assays are needed to assess the efficacy of cell replacement therapies. Mouse models that are engineered to accept human cells or non-human primate models for safety testing are of particular importance. The development of appropriate animal models for testing potential cell replacement therapies is a critical step before human therapies can be realized.

- *Determine if multipotent cells from fetal and adult tissue could be viable sources for beta cell replacement therapy.*

Many potential sources of cells could be used as a starting point for generating pancreatic islets. These sources could include cells from pig, human amniotic cells, and adult human multipotent cells isolated from bone marrow, liver, pancreas, or gut. Additional research is needed to determine which cell type(s) could be the most therapeutically useful sources.

Research Objective—Understand How Mature Beta Cells Are Maintained and Replenished in the Adult Pancreas:

- *Determine the mechanism by which beta cell number is restored after beta cell loss.*

A new challenge in beta cell research is to understand aspects of "islet maintenance," in particular, how an islet regulates its mass. Assessing beta cell mass and turnover quantitatively will require the development of better assays. The generation of monoclonal antibodies to cell surface markers on mature islet cell types, including beta cells, will aid in the development of new imaging methodology to assess beta cell mass *in vivo*. Researchers should investigate in animal models whether proliferation of existing beta cells, or alternatively, the proliferation of other cells in the pancreas, contributes significantly to the formation of new beta cells.

- *Identify factors and agents for enhancing beta cell division or decreasing cellular apoptosis.*

Testing the ability of hormones and peptides to increase beta cell proliferation and/or induce autologous beta cell regeneration should be systematically explored in animal models, both in rodents and in large animal models, such as pigs and non-human primates. High-throughput screens could be developed to identify combinations of growth factors or agents that enhance beta cell growth. A project has been initiated to develop immortalized human pancreatic beta cell lines that are functionally equivalent to primary beta cells. The transplantation of human beta cells derived from these lines into appropriate type 1 diabetes animal models will be an important first step toward the development of novel cell therapies. Investigators should determine why there are functional differences between isolated beta cells and the beta cells located within a pancreatic islet. Understanding the basic mechanisms that regulate and maintain beta cell number and

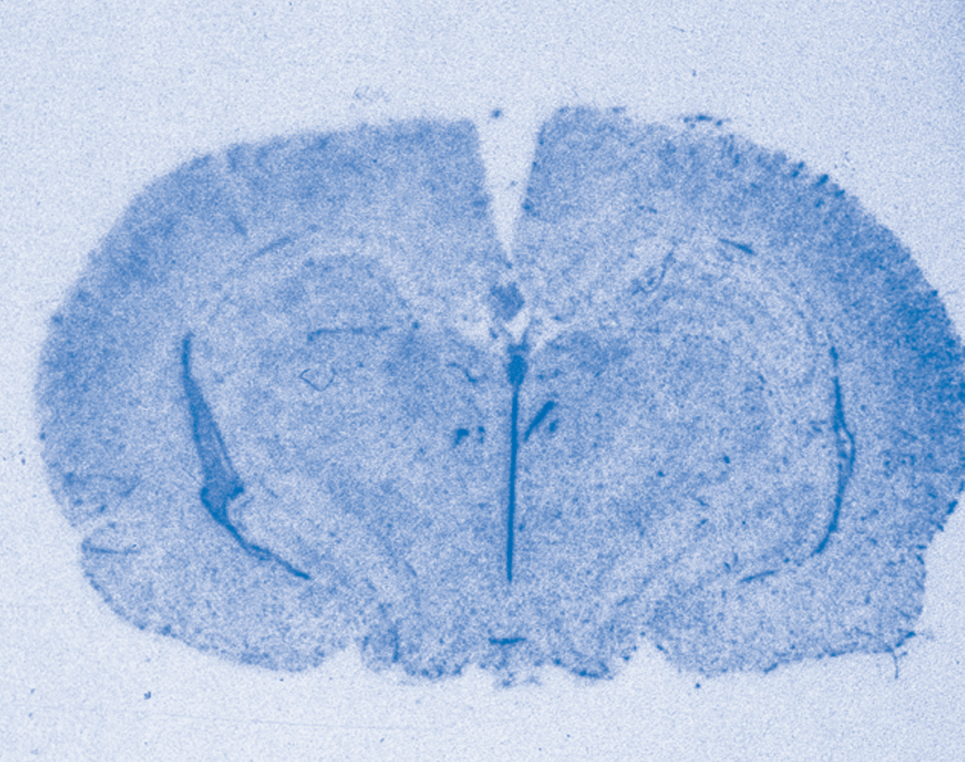
function could lead to strategies for preserving and/or restoring lost beta cells in individuals whose cells have come under attack by their own immune systems.

Research Objective—Develop Strategies To Regenerate Beta Cells Through Replication or Neogenesis:

- *Enhance understanding of the regenerative potential of beta cells.*
- *Determine whether beta cell replication or neogenesis is a clinically significant process.*
- *Develop therapeutic strategies to promote beta cell regeneration.*

Clinical research studies have demonstrated that even patients with long-standing type 1 diabetes still have some remaining beta cells in their pancreata. Furthermore, recent research has shown that both humans and animals have some ability to regenerate beta cells. Therefore, a possible therapeutic approach for treating type 1 diabetes is to promote regeneration of patients' remaining beta cells. Research is needed to fully understand the regenerative potential of beta cells before it can be determined whether *in vivo* regeneration could be explored as a potential therapeutic approach. It is also possible that, with additional insights into beta cell regeneration, islets isolated from human pancreata could be coaxed to form more beta cells in the laboratory, which would be another approach to increasing the number of islets available for transplantation. The knowledge gained through studies of the underlying molecular mechanisms of beta cell development and function, as described previously, is essential to developing strategies for the regeneration of beta cells and may also provide clues for increasing the beta cell mass in people with type 1 and type 2 diabetes.

Figure Legend: Glucose must cross the blood-brain barrier to be used by the brain as fuel. Transport of glucose across the barrier is mediated by a protein called GLUT1. Analyses of GLUT1 gene expression in brain sections from rats have shown that GLUT1 is expressed at higher levels in the brains of hypoglycemic animals. *(Image courtesy of Dr. Ian Simpson and reprinted with permission from Simpson IA, et al. J Neurochem. 72: 238-47, 1999. All rights reserved.)*



GOAL IV:

PREVENT OR REDUCE HYPOGLYCEMIA IN TYPE 1 DIABETES

Recent Scientific Advances

Brain and Peripheral Metabolic Sensing
Hypoglycemia-Associated Autonomic Failure (HAAF)
Adaptive/Maladaptive Brain Responses
Development of Animal Models of Hypoglycemia
Insulin Analogues and Glucose-Sensing Technology
Blood Glucose Awareness Training (BGAT)

Research Objectives and Strategies To Achieve Goals

Brain and Peripheral Nervous System Mechanisms of Hypoglycemia

- Define the Mechanisms and Modulators of Metabolic Sensing
- Elucidate Brain Alterations in Response to Hypoglycemia
- Develop New Strategies To Prevent or Reverse HAAF

Clinical Interventions To Prevent or Reduce Hypoglycemia

- Control Hypoglycemia Through Behavioral Therapies
- Close the Loop: Develop the Tools Required for an Artificial Pancreas

STRATEGIES TO PREVENT OR REDUCE HYPOGLYCEMIA IN TYPE 1 DIABETES

INTRODUCTION AND BACKGROUND

Although insulin therapy is the cornerstone of type 1 diabetes management and prevention of disease complications, excessive treatment with insulin can result in hypoglycemia. Too much insulin in the blood causes glucose levels to fall dangerously below a minimal threshold required to fuel the body's activities, particularly the brain. Even with newer forms of insulin that may decrease this risk, hypoglycemia remains an extremely serious, life-threatening concern.

The potential for hypoglycemic episodes has limited the use of intensive insulin therapy protocols that are known to reduce the risk of longer-term diabetic complications, such as eye, heart, and kidney disease. The immediate effects of hypoglycemia can be severe, including changes in cardiovascular and central nervous system function, cognitive impairment, increased risk for unintentional injury, coma, and death. Reducing the risk and impact of hypoglycemia would profoundly improve the quality of life for patients with type 1 diabetes.

Normally, a drop in blood glucose triggers the body's warning system to release stress hormones, including adrenaline, and to stimulate a part of the nervous system that raises glucose and results in symptoms such as shaking and sweating. The body also compensates with other "counter-regulation" defense measures, including the release of glucagon, a hormone that elevates blood glucose. However, in type 1 diabetes, glucagon release does not occur, and hypoglycemia warning signals often are not triggered due to an impaired adrenaline and nervous system response. The individual does not recognize and, therefore, cannot correct for the low blood glucose (hypoglycemia unawareness). Patients, especially children, are particularly vulnerable to hypoglycemia unawareness while they are asleep. Therefore, "nocturnal hypoglycemia" is a primary concern and the source of many anxious nights for parents of children with type 1 diabetes, who stay awake to check on the well-being of their children. The autonomic nervous system, which controls the activation of counter-regulation and warning-signal hormones that prevent hypoglycemia, becomes progressively impaired in type 1 diabetes, in large part possibly because of brain alterations that occur during each hypoglycemic episode. This vicious cycle of recurrent hypoglycemia is referred to as hypoglycemia-associated autonomic failure, or HAAF.

The widespread introduction and use of reliable, accurate, and relatively user-friendly self-monitoring glucose devices and portable insulin pumps have transformed the management of type 1 diabetes in the past two decades. Yet, because current therapy still requires painful finger sticks and injections, and in most cases still fails to achieve target glucose levels, it is critically important to develop noninvasive, continuous-monitoring, and improved insulin delivery technologies. In addition, designing a "closed-loop" delivery system or artificial pancreas—made by combining the glucose sensor and insulin pump—is the next important step in achieving close glucose control, until islet or beta cell replacement therapy becomes a viable option for patients with type 1 diabetes.

RECENT SCIENTIFIC ADVANCES

The phenomenon of hypoglycemia unawareness was originally described decades ago with the introduction of exogenous insulin. However, only recently have scientists begun to understand the complicated psychological, behavioral, and molecular mechanisms involved. This research, coupled with technological improvements in insulin therapy and behavioral interventions, is reducing the burden of hypoglycemia in type 1 diabetes.

Brain and Peripheral Metabolic Sensing: Maintenance of normal glucose balance (homeostasis) not only depends on the pancreas to release hormones in response to glucose levels, but also requires the communication of signals from all over the body to the brain, as well as from the brain itself. In the past decade, significant advances have revealed where and how the brain measures the body's metabolic status.

To measure glucose levels in the blood, cells with specialized molecular sensors—some of which are similar to those used by the pancreas—line vessels that lead to the liver and brain, as well as the gastrointestinal tract. These peripheral sensors are linked to groups of specialized glucose-sensing nerve cells (neurons) that are localized within a distributed, interconnected network within the brain, including the hypothalamus, forebrain, and hindbrain. To help the brain integrate different signals, some of these same brain neurons also respond to a variety of metabolic substrates (e.g., lactate, ketone bodies, fatty acids) and hormones (e.g., insulin, leptin, corticotropin releasing hormone), which are involved in the control of metabolism in the body. Identifying molecules and pathways for metabolic sensing may lead to targeted drug development to reduce hypoglycemia.

Hypoglycemia-Associated Autonomic Failure (HAAF): Why do most patients with type 1 diabetes suffer from recurrent bouts of hypoglycemia? The reasons became clear with the discovery that treatment-induced hypoglycemia reduces defenses against subsequent hypoglycemic episodes. Researchers have long recognized that patients with type 1 diabetes do not secrete glucagon in response to hypoglycemia, despite their ability to secrete glucagon under other circumstances. Recent findings suggest that a decrease in intraislet insulin is necessary for glucagon secretion, a response that does not occur in these patients. Identification and characterization of defects in the adrenaline warning system and autonomic response that commonly appear as insulin therapy is intensified (HAAF) have contributed to the understanding of hypoglycemia unawareness, and have laid the groundwork for future treatment.

Adaptive/Maladaptive Brain Responses: Therapies designed to protect the brain from injury due to hypoglycemia require a basic understanding of brain fuel usage and its adaptation to recurrent episodes of hypoglycemia. Recent progress has revealed how glucose and other fuels are transported into the brain despite a blood-brain barrier that blocks most molecules from entry. Surprisingly, new measurements show that glucose levels bathing the brain are only 25 percent of those in blood, which indicates that the brain's glucose supply is very tenuous, particularly during hypoglycemia. Recent studies in rodents suggest that glucose transport into the brain may be increased by prior exposure to hypoglycemia and that brain glycogen (starch) may serve as a short-term fuel reserve to partially protect the brain from injury. Studies in patients suggest that the brain may more efficiently use other (non-glucose) fuels to meet its energy needs. Ironically, while these mechanisms do partially protect the brain from being damaged by impending hypoglycemia, they attenuate the ability of patients to actually recognize and respond to hypoglycemia quickly (i.e., before dangerously low glucose levels impair brain function). With a variety of complex factors modifying cognitive outcomes in patients, it is not yet fully clear how type 1 diabetes and insulin treatment alter sensitivity of the brain to hypoglycemia.

Development of Animal Models of Hypoglycemia: The neural systems that sense and respond to hypoglycemia are localized in brain areas and peripheral organs not readily accessible for study in human beings. For this reason, the development of animal models has been critical for understanding how the brain detects and responds to single and repeated bouts of hypoglycemia. These models mimic many of the same neural, hormonal, and behavioral deficits seen in humans and are beginning to yield important new information about the body's adaptation to recurrent hypoglycemia.

Insulin Analogues and Glucose-Sensing Technology: Use of intensive insulin therapy to achieve near-normal averages for long-term blood glucose control delays the development of vascular complications of diabetes, but at the cost of a three-fold increase in the risk of severe hypoglycemia. The frequency and potential consequences of severe hypoglycemia are much greater in children than in adults. The development and widespread use of new forms of insulin have advanced the ability to provide more physiological insulin replacement. The insulin analogues, to some extent, have reduced the incidence of hypoglycemia and, coupled with new technologies available for blood glucose measurements, have made it easier for patients to manage their blood glucose. In addition, the recent introduction of continuous glucose monitoring systems to guide insulin replacement is potentially one of the most important recent advances in the treatment of type 1 diabetes because it opens the pathway to development of an artificial endocrine pancreas.

Blood Glucose Awareness Training (BGAT): Research has demonstrated that behaviors can be taught that can significantly reduce the occurrence and magnitude of hypoglycemia. BGAT is a psycho-educational program developed for patients with type 1 diabetes. It focuses on improving patients' recognition and management of extreme blood glucose levels. Analyses of the effects of BGAT have demonstrated dramatic benefits. BGAT was found to improve patients' ability to: detect both high and low blood glucose levels; judge whether they should increase or decrease their blood glucose levels; and judge whether or not they could

drive. The training program also resulted in reduced episodes of severe hypoglycemia and diabetic ketoacidosis; decreased numbers of automobile accidents; reduced fear of hypoglycemia; and improved quality of life. Because the fear of hypoglycemia is a severe limitation to the practice of intensive insulin therapy, this type of training not only can greatly benefit patients in the short-term, but also can lead to long-term benefits by permitting them to more effectively control their blood glucose levels to prevent the development of disease complications.

RESEARCH OBJECTIVES AND STRATEGIES TO ACHIEVE GOALS

The dysfunctions that lead to hypoglycemia derive from the effects of diabetes and insulin therapy on the brain. Therefore, to prevent or reduce hypoglycemia, research must focus on how the brain measures glucose levels, how it adapts to hypoglycemic events, and how neural pathways are involved in hypoglycemia unawareness and autonomic failure. Furthermore, a major research objective is to develop clinical interventions that enable patients to better control their insulin levels.

Brain and Peripheral Nervous System Mechanisms of Hypoglycemia

For the nervous system to recognize if blood glucose levels are too high or too low, specialized cells both within brain tissue and in other tissues, such as the gastrointestinal tract and pancreas, must be able to detect small changes in blood glucose. Metabolic sensing is a new and burgeoning area of basic research that is critical for the development of therapeutic interventions for the prevention and treatment of hypoglycemia. In addition to characterizing the specific cellular mechanisms involved in glucose sensing, scientists will need to understand how the neural responses to glucose levels are modulated by factors in the blood, such as hormones and metabolites, and how sensing mechanisms are integrated and signals are relayed to different areas of the brain and peripheral tissues. Localization of these brain areas and neural networks in humans can be achieved through improvements in the resolution and sensitivity of imaging technologies (see Goal VI).

Progress in hypoglycemia research depends on understanding not only brain and peripheral metabolic sensing, but also brain alterations in response to hypoglycemia and mechanisms underlying HAAF. HAAF occurs as a result of episodes of hypoglycemia that commonly occur during insulin therapy. These episodes suppress and lower the level of glucose at which subsequent counter-regulatory responses occur, particularly the release of the hormone adrenaline. Adrenaline is the critical defense against hypoglycemia for type 1 diabetes patients because they already lack natural insulin and glucagon responses to hypoglycemia. Furthermore, suppression of adrenaline and sympathetic nervous system responses reduces the symptoms of low blood glucose, such as increased heart rate, sweating, and a desire to eat. These warning symptoms alert patients to take corrective action.

HAAF can be reversed by as little as several weeks of scrupulous avoidance of hypoglycemia. Unfortunately, this is extremely difficult to accomplish in clinical practice without causing deteriorating glucose control—an undesirable consequence with respect to diabetes complications. Thus, it is of critical importance to define the currently unknown mechanisms responsible for HAAF, so that new clinical strategies could be developed to prevent, correct, or compensate for HAAF, while at the same time, improving blood glucose control.

Research Objective—Define the Mechanisms and Modulators of Metabolic Sensing:

- *Identify and elucidate the mechanisms involved in glucose sensing in the brain.*

Multiple mechanisms involved in sensing blood glucose concentrations are being discovered. The same enzyme that pancreatic islets use to measure glucose (glucokinase) may play a role in the brain. Recent evidence suggests that glucose sensors use chloride or ATP-sensitive potassium ion channels, which increase or decrease the electric state of brain cells by allowing charged ions to pass in response to binding a signaling molecule. The enzyme that muscle and other tissues use to sense fuel deficits (i.e., AMP-kinase) may also be used by the brain sensors.

Glucose-sensing mechanisms are also evident in peripheral tissues, including the pancreas, liver, carotid body, and gastrointestinal tract. Thus, further characterization of glucose-sensing tissues in the periphery may provide a model for understanding brain mechanisms. Identification of the molecular mechanisms involved in glucose sensing and how they are altered by diabetes, its treatment, and previous exposure

to hypoglycemia will facilitate the development of pharmacological agents targeted for hypoglycemia prevention.

- *Determine the hormonal and metabolic modulators involved in glucose sensing.*

Insulin, the hormone that causes hypoglycemia, may also alter the responsiveness of glucose-sensing mechanisms and, ultimately, the responses of the brain to hypoglycemia. Circulating peripheral insulin gains access to the brain and elicits changes in release of neuropeptides and neurotransmitters. Similarly, other metabolic hormones (e.g., leptin, corticotropin-releasing hormone), fuels (e.g., short- and medium-chain fatty acids), or nitric oxide have been suggested as modulators of glucose-sensing mechanisms. Finally, glucose sensing might be modulated by changes in the number of glucose transporters—molecules that recognize glucose outside the cell and bring it inside; or altered regulation of cellular receptors—molecules on the surface of cells that take up hormones and neurotransmitters so that they can do their work in the body.

In addition to nerve cells, the brain contains other types of specialized cells that may play a critical role during hypoglycemia. Specifically, glia (non-neuronal supporting cells within the brain) have the capacity to provide a source of energy to neurons during hypoglycemia by supplying both lactate (an energy-yielding breakdown product of glucose) and glycogen (a form of stored glucose arranged in long chains that can be broken down when needed). Future studies will need to determine if altered glucose sensing in diabetes or following hypoglycemia might be mediated by changes in glial function. In keeping with this possibility, glial-derived lactate has been reported to act as a signaling molecule in glucose-sensing neurons.

Research Objective—Elucidate Brain Alterations in Response to Hypoglycemia:

- *Determine alterations in brain metabolism and function induced by recurrent hypoglycemia.*

The brain appears to respond to the stress imposed by insulin-induced hypoglycemia by using mechanisms to ensure a continued supply of energy and protect the brain from damage. Varied scientific approaches are required to define how metabolism in the brain changes or adapts to reduce the insult and injury from recurrent episodes of hypoglycemia. A first step is to establish animal models of hypoglycemia in diabetes that replicate adaptive mechanisms of the brain. This resource will enable researchers to test therapeutic agents, isolate brain sections in culture, and apply newly developed gene array technologies directly to brain tissue, thereby facilitating identification of new protective mechanisms. Animal models would also be useful for protocols not ethically possible in humans, such as examining if the level of glucose control in diabetes modifies the brain's metabolic responses to recurrent hypoglycemia.

The relative importance of the mechanisms involved in the brain's attempt to protect itself from injury must be established so that, ultimately, they may be targeted for therapeutic intervention. Potential mechanisms to investigate include: increased transport of glucose into the brain; increased storage of glucose as glycogen; and alternative fuel utilization (e.g., lactate, fatty acids), as occurs normally during starvation. Approaches to these questions can be tested clinically with new state-of-the-art brain imaging technologies, particularly positron emission tomography (PET) and magnetic resonance spectroscopy (MRS).

Of significant clinical concern is the effect of recurrent hypoglycemia on cognitive function. While it is known that cognitive function is impaired by acute hypoglycemia, the impact of intensive insulin therapy and hypoglycemia unawareness on cognition is poorly understood, in part because of the limitations of standard cognitive testing procedures. This issue can be addressed clinically with sensitive tools, such as functional magnetic resonance imaging (fMRI). This technology allows scientists to directly image the local activity within patients' brains while they are performing cognitive tests under conditions of hypoglycemia. Finally, insulin itself could alter cognition. Thus, studies need to determine the contribution of insulin per se to the changes in cognition seen during insulin-induced hypoglycemia.

- *Prevent hypoglycemia-induced brain injury and promote protective adaptations.*

A major goal of uncovering both the adaptive and maladaptive mechanisms occurring in response to hypoglycemia is to develop therapies to reverse brain damage. Studies using state-of-the-art imaging technologies to assess brain function and metabolism could be performed in patients in whom hypoglycemic events are virtually eliminated, such as patients treated with an artificial pancreas or an islet transplant. These patients would be compared to type 1 diabetes patients with and without frequent episodes of hypoglycemia and hypoglycemia unawareness. Understanding the natural protective adaptations of the brain could lead to interventions to further promote protection from injury, as well as maintenance of cognitive performance during hypoglycemia. For example, if the brain protects itself by increasing its use of alternative

fuels, patients could take oral supplements to augment their supply of these fuels. If the brain burns glycogen during hypoglycemia, patients could take agents that promote glycogen storage.

▸ *Identify potential genes involved in individual susceptibility to hypoglycemia.*

Little is known about the potential influence of genetic risk factors in patients who suffer from frequent episodes of severe hypoglycemia. Angiotensin converting enzyme (ACE) may play a role because high ACE activity and the presence of the D allele of the ACE gene are known to predict a high rate of severe hypoglycemia in type 1 diabetes. However, further genetic screening of highly susceptible patients could help identify other genes and, potentially, prevention strategies.

Research Objective—Develop New Strategies To Prevent or Reverse HAAF:

▸ *Elucidate the mechanisms of HAAF.*

Given the lack of understanding of HAAF, prevention will require experiments in animal model systems before rational approaches can be developed to enhance hormone defense systems in diabetes. Animal models afford the opportunity, at the molecular level, to determine whether hypoglycemia itself alters the function of glucose-sensing nerves, and, if so, how this occurs. For example, is it due to a change in nerve cell signaling, metabolic function of the cells, circulating or brain-derived hormones, brain neurotransmission, or a combination of some of these factors?

Furthermore, it is important to develop animal models of HAAF that not only replicate the pathophysiology of diabetes in humans, but also are consistent among laboratories. This approach will facilitate the establishment of a central repository and/or resource listing for molecular probes, cell lines, and transgenic animals for further research studies on HAAF. A greater understanding of the putative molecular mediators leading to defective hormone responses in type 1 diabetes will provide the basis for testing unique therapies aimed at reversing HAAF. These ideas would initially be explored in animal models, then eventually translated to clinical drug trials to test if the responses to acute hypoglycemia are improved and hypoglycemic risk is reduced.

▸ *Identify the clinical consequences of HAAF.*

In humans, HAAF can be examined by applying newly improved imaging technologies, such as PET, fMRI, or MRS, to study the activation and integration of specific areas of the brain that trigger hormonal defenses against hypoglycemia. Localization of region-specific activation patterns in humans will depend on continued improvements in the sensitivity of imaging techniques. These imaging studies should be performed in conjunction with functional monitoring of neuroendocrine, metabolic, and peripheral nervous system responses, as well as cognitive and behavioral responses. Integrative, multidisciplinary studies of this nature are required to investigate the broad pathological consequences of HAAF in humans. For example, recent studies demonstrate that the sympathetic nervous system's responses to hypoglycemia are reduced during sleep. Therefore, patients with type 1 diabetes are less likely to be awakened by hypoglycemia. This sleep-related HAAF, in the context of imperfect insulin replacement, probably explains the high frequency of nocturnal hypoglycemia in type 1 diabetes. Further studies are required to directly assess the impact of prior hypoglycemia at night on the counter-regulatory and neural responses during both sleep and exercise.

▸ *Develop and test therapies to restore counter-regulation.*

The complete loss of glucagon response to hypoglycemia develops in nearly all type 1 diabetes patients within a few years of diagnosis, independent of HAAF. Undoubtedly, if the glucagon response to hypoglycemia could be restored, the problem of hypoglycemia would be greatly minimized. Current data suggest that the inability of patients to suppress their intraislet insulin secretion may, in part, explain the absent glucagon response. Therefore, mechanistic studies characterizing the glucagon defect at the whole organ, cellular, and molecular levels would have great potential benefit for the development of agents to overcome the glucagon defect, because the alpha cell works normally in type 1 diabetes patients at other times.

Clinical Interventions To Prevent or Reduce Hypoglycemia

The frequency of hypoglycemia in patients with diabetes depends on many factors, including patients' management of blood glucose levels, training and ability to recognize the circumstances that precede hypoglycemia, and other individual differences. Behavioral and clinical strategies can be tailored to the individual needs of patients to prevent or reduce hypoglycemic episodes.

Despite improvement in clinical management, treatment of type 1 diabetes with exogenous insulin replacement will not be optimal until there is feedback control of insulin delivery, accomplished either by beta cell replacement or by a mechanical, closed-loop insulin delivery system based on

continuous glucose monitors (CGMs). Children and adolescents are particularly vulnerable to hypoglycemia. Therefore, they are an ideal target population for closed-loop insulin delivery, because they are likely to receive the greatest benefit from it and are not appropriate candidates for islet transplants or other experimental approaches that currently involve lifelong immunosuppressive therapies.

The development of accurate and reliable CGMs is the first step toward closed-loop insulin delivery. In comparison to the mature home glucose meter technology that has benefited from 25 years of development, CGM technology is still in its infancy and needs further refinement. As has been demonstrated by the Diabetes Research in Children Network (DirecNet), the first generation devices approved by the FDA have major limitations.

Progress toward development of an artificial pancreas is likely to be a stepwise, iterative process. Several new and improved "real-time" CGM systems have recently been introduced, and may be followed by the development of algorithms that allow for appropriate insulin delivery via continuous delivery systems.

Research Objective—Control Hypoglycemia Through Behavioral Therapies:

- *Refine and link behavioral interventions and algorithms that predict risks of hypoglycemia.*

Hypoglycemia is not a homogenous phenomenon that affects every patient with diabetes in the same way; some patients are more vulnerable to morbidity and mortality. For example, recent research has shown that patients with recurrent driving mishaps have particular biological and behavioral characteristics (e.g., exhibiting enhanced insulin sensitivity, greater cognitive and motor decay with equivalent levels of hypoglycemia, and other characteristics such as being more likely to live alone). Clinical research that maps patients' behavioral traits could assist the development of algorithms that predict the risk of adverse hypoglycemic events. These models could be coupled with interventions that reduce the risk in identified patients (e.g., preventing driving mishaps). Behavioral research could help patients improve the recognition and prevention of hypoglycemia.

- *Evaluate behavioral approaches to preventing nocturnal hypoglycemia.*

Nocturnal hypoglycemia remains a significant clinical problem and source of concern for parents of children with type 1 diabetes. A practical aid to them in helping their children maintain normal blood glucose levels would be a systematic evaluation of the impact of the dietary composition of evening meals and snacks to reduce the incidence of nocturnal hypoglycemic events and to improve subsequent morning glucose values.

Research Objective—Close the Loop: Develop the Tools Required for an Artificial Pancreas:

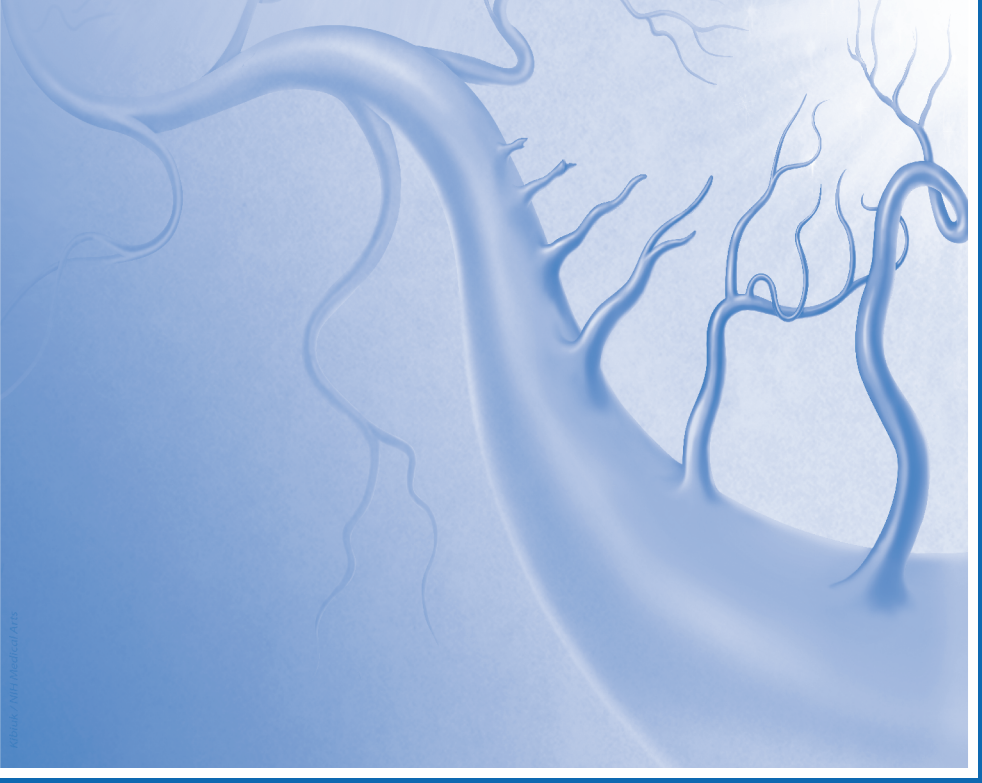
- *Optimize use of continuous glucose monitors.*

Currently approved CGMs are limited in their ability to reliably detect low blood glucose. Reliable identification of hypoglycemia in the home setting would minimize the risks associated with hypoglycemic events and permit the evaluation of factors, such as exercise and diet, that influence the risk of hypoglycemia, especially during the night. Once reliable sensors are developed, it will be possible to conduct clinical trials of real-time glucose sensor monitoring with outcome measures, including reducing the risk of hypoglycemia, lowering HbA1c levels, and enhancing counter-regulatory hormone responses using insulin pumps or basal/bolus injection regimens. As part of these investigations, guidelines could be developed on how to use glucose sensor data to optimize glucose control and minimize the risk of severe hypoglycemia.

- *Develop algorithms needed to link glucose monitors with insulin delivery.*

The development of computerized algorithms that automatically vary insulin delivery rates based on glucose sensor data is essential to providing adequate control of postprandial hyperglycemia and eliminating the risk of hypoglycemia. Further studies would test how robust such systems are over time and under a variety of real-life conditions. Once reliable sensors are developed and appropriate algorithms are established, their integration into a closed-loop system would be feasible. The development of closed-loop insulin delivery would initially start with external, minimally invasive, subcutaneous, short-term sensors and external pumps, and would lead ultimately to fully implantable, long-term systems. This progress will require cooperation among the NIH, industry, and private foundations. Moreover, it will require enhanced collaborations among diabetes specialists, islet physiologists, bioengineers, and the computational biology/informatics community.

Figure Legend: Angiogenesis, the branching and extension of existing blood vessels, is integral to development of the blood vessel system (vasculature) in both the embryo and adult. Some diabetes complications (e.g., nerve damage) may be treated by *stimulating* angiogenesis, whereas other complications (e.g., retinopathy) may be treated by *inhibiting* angiogenesis.



GOAL V:

PREVENT OR REDUCE THE COMPLICATIONS OF TYPE 1 DIABETES

Recent Scientific Advances

Progress in Reducing Diabetic Nephropathy

Role of Reactive Oxygen Species (ROS) in Complications Pathogenesis

Therapeutic Approaches Based on a Soluble Form of the Receptor for Advanced Glycation Endproducts (RAGE)

Insulin Resistance–An Independent Cardiovascular Risk Factor in Type 1 Diabetes

Diabetic Heart Damage from Altered Fuel Metabolism

Impaired Blood Vessel Formation from Bone Marrow Progenitor Cells in Diabetes

Sustained Effect of Glycemic Control on Complications Susceptibility–"Metabolic Memory"

Behavioral Interventions Improve Metabolic Control

Research Objectives and Strategies To Achieve Goals

Molecular Mechanisms of Common Pathways in Diabetic Complications

- Identify Molecular Pathways of Hyperglycemia Damage
- Clarify Mechanisms Linking Fuel Utilization and Heart Disease
- Understand the Systems Biology of Diabetic Complications

Metabolic Memory

- Discover the Molecular Mechanisms of Metabolic Memory

Genetic Factors

- Identify Genes Conferring Susceptibility and Resistance to Diabetic Complications

Animal Models

- Develop More Human-like Animal Models of Diabetic Complications

Biomarkers and Surrogate Endpoints To Facilitate Clinical Trials

- Identify Biomarkers or a Combination of Biomarkers for Earlier Detection of Cell and Tissue Damage
- Validate Surrogate Endpoints for Assessing the Progression of Complications in Clinical Trials

Therapies To Improve Patient Health

- Identify Therapeutics That Prevent or Reverse the Development and Progression of Diabetic Complications
- Mitigate Psychosocial Complications and Comorbidities of Diabetes To Improve Quality of Life
- Combine New Technology for Diabetes Management with Behavioral and Translational Research

STRATEGIES TO PREVENT OR REDUCE THE COMPLICATIONS OF TYPE 1 DIABETES

INTRODUCTION AND BACKGROUND

For a child at the beginning of the 20th century, the diagnosis of type 1 diabetes was equivalent to a death sentence. The discovery of insulin commuted that death sentence, but it soon became apparent that insulin treatment allowed many of these children to live just long enough to develop diabetes-induced blindness, kidney failure, and coronary disease. At the beginning of the 21st century, type 1 diabetes still has a profound effect on many people's lives, because these long-term health complications that affect nearly every organ system in the body still occur with alarming frequency.

Patients with type 1 or type 2 diabetes face the possibility of similar complications. For patients and their families, the following grim statistics about complications are far too familiar.

- Life expectancy may be shortened by about 15 years (4), with premature deaths due primarily to heart attacks and strokes (5). Rates of cardiovascular disease are increased up to 10-fold compared to people in the age-matched general population (2, 3). In addition, diabetes impairs repair pathways necessary for the success of established cardiovascular therapies, such as coronary angioplasty, bypass grafting, and lower extremity revascularization. This causes a significantly lower success rate in treating vascular diseases in patients with diabetes.

- In addition to injuring the large vessels of the heart and brain, diabetes damages the small blood vessels of the body, called the microvasculature. Diabetes (type 1 and type 2) is now the leading cause of new blindness in people 20 to 74 years of age (5). Retinal damage (retinopathy) is detectable in virtually all patients with type 1 diabetes after 20 years, as a consequence of damage to the microvessels of the retina.

- Diabetes also leads to protein in the urine (microalbuminuria) and eventually to irreversible kidney disease (nephropathy), which progresses to end-stage renal disease, requiring dialysis or kidney transplantation. Importantly, only about one-third of patients with type 1 diabetes appear to be susceptible to this devastating complication (20). The factors that determine susceptibility or resistance to nephropathy are still unknown.

- More than 60 percent of patients with diabetes are affected by painful nerve damage and loss of sensation (neuropathy), particularly in the legs and feet (5). Foot ulcers often arise because of the inability to perceive pain, and then fail to heal because of insufficient blood flow and other factors secondary to diabetes. Amputation of the lower extremities is too frequently the end result of nonhealing ulcers. Diabetic nerve damage also contributes to erectile dysfunction, urinary incontinence, and nocturnal diarrhea.

- Depression severe enough to warrant intervention is increased in patients with type 1 diabetes. Conventional antidepressant treatments are effective in the presence of diabetes, but with discontinuation of treatment, recurrence of depression is common and usually accompanied by deterioration in glycemic control.

- Other complications of type 1 diabetes include increased rates of birth defects in children of mothers with diabetes and severe periodontal disease.

Preventive strategies are beginning to reduce the incidence of diabetes complications. Nonetheless, tight control of blood glucose levels is difficult to achieve because of the risk of hypoglycemia and the need for unrelenting vigilance about dietary intake, the monitoring of blood glucose levels, and the administration of insulin. Good control of blood pressure and blood lipid levels—as well as careful monitoring for retinal damage, albumin in the urine, sores on the feet, and other signs and symptoms of diabetes—are all measures for preventing or mitigating complications of diabetes. Although blockade of the renin-angiotensin system is well-established to slow the progression of diabetic nephropathy, it does not always prevent the development of renal failure. The treatment options for other complications are even less satisfactory. In particular, symptomatic treatment for nerve pain is poor. While laser photocoagulation has been an extremely

important advance for treating diabetic retinopathy, vision loss from this complication is not always preventable, even with the best interventions.

In type 1 diabetes, complications are due to metabolic derangements caused by loss of insulin resulting from the autoimmune destruction of insulin-producing beta cells. The best understood metabolic defect causing diabetic complications is hyperglycemia, which can modify the extracellular environment and, in some cell types, directly lead to excess glucose inside the cell. Intracellular hyperglycemia initiates a cascade of changes in cell metabolism that includes increased production of reactive oxygen species, increased levels of sugar-modified proteins, and activation of a number of signaling pathways. In addition to causing hyperglycemia, insulin deficiency can contribute to end-organ damage in type 1 diabetes through alterations in the metabolism of lipids and lipoproteins. In the endothelial cells that line the blood vessels in the eye, kidney, nerve, and heart, these alterations lead to increased leakiness of blood vessels, decreased vascular density, inadequate delivery of blood due to cell loss, and altered expression of cell-surface proteins that initiate and perpetuate a damaging inflammatory response. Tissue responses to these cellular changes in the blood vessels are specific for each tissue and organ, with genetic variation playing an important role in determining the nature and extent of these responses for each individual.

Advancing scientific knowledge of the physical and emotional complications of diabetes to improve clinical care is a multidimensional challenge. Meeting this challenge would have overwhelming benefits for people with type 1 diabetes. New discoveries about diabetic complications may also improve the lives of millions of Americans with type 2 diabetes, who also suffer from the same devastating complications.

RECENT SCIENTIFIC ADVANCES

Significant progress in understanding diabetic complications has occurred in the past decade. These discoveries are leading to the development of effective therapies to prevent and treat the cell, tissue, and organ damage caused by diabetes.

Progress in Reducing Diabetic Nephropathy: Recent reports indicate that prevention efforts are beginning to have dramatic effects on the rates of diabetic nephropathy in patients with type 1 diabetes. This devastating complication of diabetes has historically been seen in as many as one-third of individuals with diabetes after 20 or 30 years of disease (20). In the most recent population-based study from Finland, however, only 7.8 percent of patients with type 1 diabetes have renal failure after 30 years of diabetes (21). Declines in the incidence of end-stage renal disease due to diabetes are being noted for the U.S. population as well, in reports from the United States Renal Data System. These gains are most noteworthy in diabetic patients under age 30 (most of whom have type 1 diabetes) and are restricted to Caucasians and not observed in African Americans. The rate of end-stage renal disease in Caucasians under 30 with diabetes is nearly half the rate seen in the late 1980s and early 1990s (9). Since that time, several clinical strategies have been proven to significantly reduce the progression of diabetic nephropathy. These include angiotensin-converting enzyme inhibitors (ACE inhibitors) and angiotensin receptor blockers (ARBs), which lower protein in the urine and are thought to directly prevent injury to the kidneys' blood vessels; and careful control of blood glucose and blood pressure. Credit for the recent improvements likely goes to implementation in clinical practice of these strategies to prevent disease, including better glycemic control, hypertension management, and use of ACE inhibitors and ARBs. Research to build on this success and extend it to all populations in the United States is a high priority for the NIH.

Role of Reactive Oxygen Species (ROS) in Complications Pathogenesis: Over the past 35 years, several molecular mechanisms have been implicated in glucose-mediated vascular damage. Each of these mechanisms has been studied independently of the others, and there has been no apparent common element linking them. Recent discoveries have made clear that all of these seemingly unrelated mechanisms may arise from a single, hyperglycemia-induced process: the overproduction of the reactive free radical molecule, superoxide. It now appears that the energy-generating cellular organelles called mitochondria are required for the initiation of hyperglycemia-induced superoxide production, which can, in turn, activate a number of other superoxide production pathways that may amplify the original damaging effect of hyperglycemia. Increased free fatty acid oxidation in mitochondria produces superoxide as well. In diabetic mice genetically engineered to produce high levels of an enzyme that degrades superoxide (called "superoxide dismutase"), diabetes fails to activate any of the classic hyperglycemia-induced damaging pathways, and these mice do not develop

diabetic kidney disease. This advance points to the central role of a single pathway involved with complications in multiple organs. Several novel pharmacologic approaches based on this unifying mechanism have already prevented diabetic eye, kidney, and nerve pathology in rodent models of diabetes.

Therapeutic Approaches Based on a Soluble Form of the Receptor for Advanced Glycation Endproducts (RAGE): Although elevated levels of low-density lipoproteins (LDL cholesterol) appear to be required for atherosclerosis, the pathologic result for any given level of LDL depends on a wide variety of other factors, many of which are pro-inflammatory molecules secreted by a variety of tissues and cell types. Recently, it has been established that nearly all people with type 1 diabetes have accelerated atherosclerosis and coronary artery disease, which is associated with increased local tissue levels and blood levels of pro-inflammatory molecules. A major advance in this field is the identification of RAGE, a component of the innate immune system originally identified based on its ability to bind sugar-modified proteins known as advanced glycation endproducts (AGE). RAGE is found in many tissues, including those prone to diabetic complications. The binding of AGE to RAGE activates signaling pathways that lead to generation of reactive oxygen species. In addition, a soluble form of RAGE binds a number of pro-inflammatory factors, such as S100 calgranulins and high mobility group box 1 (HMGB1), thereby preventing their interaction with cellular receptors. Treatment with this soluble form of RAGE prevents accelerated atherosclerosis in several models of experimental diabetes. In addition, soluble RAGE blocks kidney disease in diabetic mice. Because soluble proteins are not ideal pharmacologic agents in all settings, especially for those that require chronic administration over many years, additional research focused on the development of orally available antagonists (i.e., drugs that block the receptor), and modification of both the inflammatory peptides and the receptor, should lead to the development of important new therapies to retard atherosclerosis in people with diabetes.

Insulin Resistance—An Independent Cardiovascular Risk Factor in Type 1 Diabetes: In some regards, the classic distinction between type 1 and type 2 diabetes is beginning to break down. Type 1 diabetes is a disease in which there is a loss of insulin production due to autoimmune destruction of insulin-producing cells. In type 2 diabetes, loss of effective insulin action is due to a combination of defects, both in normal insulin action (insulin resistance) and in the ability of pancreatic beta cells to overcome this insulin resistance by secreting enough additional insulin. Over the past 10 years, evidence has mounted to show that, in type 1 diabetes, hyperglycemia itself eventually causes secondary insulin resistance in nearly all patients. Because insulin resistance appears to operate as a significant factor in the development of cardiovascular disease, independent of other mechanisms such as lipid abnormalities and high blood pressure, the discovery that hyperglycemia causes insulin resistance in type 1 diabetes links accelerated atherosclerosis in patients with type 2 diabetes with accelerated atherosclerosis in type 1 diabetes. In addition, insulin resistance leads to a greater likelihood of heart failure following a heart attack. These observations suggest novel pharmacological approaches for reducing cardiovascular disease in patients with type 1 diabetes via direct reduction of insulin resistance.

Diabetic Heart Damage from Altered Fuel Metabolism: A significant advance in understanding diabetic heart disease is the discovery that derangements in cardiac fuel utilization are, at least in part, responsible for myocardial disease caused by diabetes. Both cardiac and skeletal muscle cells (myocytes) have high energy requirements. Cells normally use both glucose and fatty acids as energy sources. In diabetic heart muscle, insufficient insulin action reduces the ability of myocytes to use glucose as a source of energy. To compensate, these cells switch to fatty acids as their primary energy source, with chronic activation of the nuclear receptor PPAR-alpha mediating this switch. The increased flux of fatty acids into heart muscle cells overwhelms the mitochondria's abilities to burn this fuel. The result is intracellular accumulation of ROS and a variety of fatty acid metabolites, whose deleterious effects on cells have been termed "lipotoxicity." Accumulation of fatty acid metabolites leads to death of cardiac myocytes in the hearts of diabetic animals and makes these cells especially sensitive to further damage from hypoxia associated with angina or a heart attack. These findings may lead to therapies that increase glucose utilization in heart muscle and thereby reduce the damage caused by accumulation of fatty acids. They could also lead to the development of diagnostic imaging methods to detect deleterious changes in cardiac cell metabolism before they lead to heart disease.

Impaired Blood Vessel Formation from Bone Marrow Progenitor Cells in Diabetes: Diabetic complications result not only from damage to cells and tissues, but also from the inadequacy of the repair process. During the acute response to injury, new blood vessel growth rescues "stunned" areas of the heart or central nervous system, reducing morbidity and mortality. With chronic low perfusion, the development of collateral vessels reduces the size

and severity of a subsequent infarction. Circulating progenitor cells from the bone marrow promote the regeneration of blood vessels by acting in concert with the cells and extracellular matrix at the site of injury. A major advance is the observation that these endothelial progenitor cells are depleted and dysfunctional in diabetes, and that injection of normal progenitor cells can improve blood supply to the tissues and nerve function in experimental diabetes. Research focused on the diabetes-induced impairment of this process could lead to novel drug- and cell-based therapies for people with diabetes to restore compensatory vessel formation in cardiovascular disease, stroke, peripheral vascular disease, and wound healing. In the diabetic retina, however, overly exuberant vascular repair processes can result in excessive proliferation of small vessels. Molecular pathways responsible for the new vessel growth have been identified, and this work suggests new molecular targets for drugs that could protect the retina.

Sustained Effect of Glycemic Control on Complications Susceptibility—"Metabolic Memory": In 1993, the results of the landmark Diabetes Control and Complications Trial (DCCT) showed that, in people with short-duration type 1 diabetes, intensive glycemic control dramatically reduced the occurrence and severity of diabetic microvascular complications. After the announcement of the DCCT results, many patients who had been in the standard therapy group adopted more intensive therapeutic regimens, and their level of glycemic control improved, as measured by the hemoglobin A1c (HbA1c) test. At the same time, the mean level of HbA1c worsened for patients who had been in the intensive therapy group. The post-DCCT HbA1c values for both groups have become nearly identical during the approximate 10 years of follow-up in the ongoing Epidemiology of Diabetes Interventions and Complications Study (EDIC).

Surprisingly and provocatively, however, the effects of a 6.5-year difference in HbA1c during the DCCT on the incidence of retinopathy and nephropathy have persisted, and have even become greater over the subsequent decade of follow-up. People in the standard therapy group continued to have a higher incidence of complications, even with an improvement in glycemic control during the EDIC. In contrast, people in the intensive therapy group continued to have a lower incidence of complications, even with a worsening of glycemic control during EDIC. In addition, early intensive glycemic therapy was recently shown to markedly reduce later development of atherosclerotic changes, heart attacks, and strokes.

The phenomenon that glycemic control could have long-lasting effects, called metabolic memory, elicits a number of questions: How can a finite period of good or bad glycemic control have such long-lasting effects? Is there a point in the development of complications in which the progression becomes relatively independent of glycemic control? The discovery of the molecular and cellular basis of metabolic memory is urgently needed, so that solutions can be designed to mimic or induce the protective "memory" of good glycemic control and to inhibit or reverse the sensitizing "memory" of poor glycemic control.

Behavioral Interventions Improve Metabolic Control: In combination with good clinical care, psychosocial interventions can improve glycemic control, leading to prevention of diabetes complications. Behavioral research has successfully tested educational strategies, coping skills training, diabetes-related stress management interventions, and behavioral counseling for patients, their families, and significant others. Children can be intensively managed without increasing hypoglycemia; family-focused intervention can yield beneficial glycemic control outcomes, particularly among patients in the poorer range of control before treatment. Research has also shown that self-care autonomy in children with diabetes is associated with adverse outcomes. To counter this, developmentally appropriate parental involvement in diabetes tasks is essential for improved metabolic functioning. There is mounting evidence that adolescents struggling with diabetes adaptation continue to have problems with control as they get older, including a higher likelihood of depression, earlier complications, and disengagement from the health care system upon graduating from pediatric care. These data suggest the importance of focusing on interventions upon diagnosis, leading to metabolic control and preserved psychosocial function. Strategies to help families manage conflict have been developed, and the goal is to find ways to broadly implement them.

The prevention and reduction of complications will be greatly facilitated by the discovery and development of agents that prevent or reverse the cellular and tissue injury induced by type 1 diabetes and hyperglycemia. Each research objective in this chapter addresses a critical area necessary to achieve this overarching goal. An understanding of the molecular mechanisms and genetic risk factors underlying diabetic complications may lead to the identification of new molecular targets for drug development. Application of the latest advances in drug development technology to diabetic complications has the potential to greatly decrease drug development time and improve prospects for clinical success. To test promising drug candidates, animal models are needed that more completely mimic the human pathology of diabetic complications. The discovery of biomarkers and surrogate endpoints for the early manifestations of diabetic complications could allow targeted therapies and potentially shorten the duration of clinical testing, thus removing a significant barrier to achieving clinically useful therapeutics for diabetic complications.

Molecular Mechanisms of Common Pathways in Diabetic Complications

Understanding of the mechanisms underlying diabetic complications has greatly expanded in recent years as scientists have identified several implicated molecules. Recent technological advances present an important opportunity to fully characterize the disease pathways that cause retinopathy, nephropathy, neuropathy, cardiomyopathy, accelerated atherosclerosis, and other diabetic complications. Basic science discoveries—such as the recent discovery of microRNAs (see Goal VI), which may regulate expression of one-third of all genes—will help researchers studying diabetic complications to identify completely new and unpredicted therapeutic targets and clinically useful biomarkers.

Research Objective—Identify Molecular Pathways of Hyperglycemia Damage:

- *Discover the factors controlling hyperglycemia-induced reactive oxygen species (ROS) formation and adaptive and maladaptive cellular responses to increased ROS.*
- *Identify the molecular events controlling RAGE expression and endogenous soluble RAGE production.*
- *Discover the mechanisms by which hyperglycemia impairs bone marrow progenitor cell function, especially vascular cell progenitors needed to repair wounds and revascularize ischemic heart muscle, peripheral nerves, and lower limbs.*
- *Identify the mechanisms of vascular proliferation in diabetic retinopathy.*
- *Discover the mechanisms leading to diabetic neuropathy that can occur through impaired blood vessel function and other causes, such as AGE formation and alterations in nerve growth factor signaling.*

The identification of the cellular and molecular pathways involved in diabetic complications provides a strong foundation for research on key regulatory steps in these pathways that should lead to exciting and clinically relevant discoveries. These breakthroughs in basic research on cellular pathways promote interdisciplinary research with investigators in other basic science and disease-based fields. The goals listed above on oxidative stress, inflammation (RAGE), and angiogenesis are central not only to diabetic complications, but are also involved in numerous other diseases, such as cancer and atherosclerosis. Therefore, research in this area will benefit from and contribute to the much broader biomedical research endeavor.

Research Objective—Clarify Mechanisms Linking Fuel Utilization and Heart Disease:

- *Characterize the factors controlling increased fatty acid accumulation and mitochondrial oxidation in the development of diabetic cardiomyopathy and accelerated atherosclerosis, as well as the mechanisms by which this cellular lipotoxicity induces cell damage.*

The heart has extraordinarily high energy requirements related to its function as a pump throughout life. The energy demands of the heart are met through a high-capacity mitochondrial system that is well suited to oxidize fatty acids and glucose to generate energy. In the insulin-deficient state, this high-capacity mitochondrial system is pushed to the limit through increased reliance on fatty acid oxidation as the energy source. Whereas the increase in cellular fatty acid utilization in the insulin-deficient state is initially an adaptive response, evidence is emerging that this increased fatty acid import and oxidative flux lead to deleterious consequences relevant to the pathogenesis of myocardial and vascular disease. Early studies in this area have identified a number of potential mechanisms linking increased fat utilization to cellular toxicity, including the accumulation of lipid products that could trigger signaling events leading to apoptosis (cell suicide); generation of ROS via increased oxidative flux through mitochondria and peroxisomes; and secondary damage to mitochondria, leading to bioenergetic abnormalities. Future studies related to each of these potential

mechanisms will be important for enhancing understanding of the pathogenesis of cardiovascular toxicity in type 1 diabetes. Moreover, identification of relevant cellular events involved in this response could pave the way for identification of new therapeutic targets and biomarkers.

Research Objective—Understand the Systems Biology of Diabetic Complications:

- *Apply a systems biology approach to research on diabetic complications.*

The pathogenesis of diabetic complications encompasses much more than the cellular responses to the metabolic defects of diabetes. Each known diabetes-induced abnormality within a cell can be thought of as connected in a circuit-like arrangement with other intracellular molecules. Similarly, the pathology in one cell type is also connected in a circuit-like arrangement with other cell types in a specific tissue, and each tissue type is likewise connected to other tissues and organ systems, with changes in one nodal component influencing many other points in the network. The emerging field of systems biology will have a major impact on progress in this area. Systems biology is a powerful, mathematically based discipline that seeks to analyze the many simultaneously occurring changes in intracellular, intercellular, and inter-organ contexts as complex, interconnected circuits that have nodal control points, much like the electronic circuits on a microchip. The use of a systems biologic approach can lead to models of *in vitro* and *ex vivo* systems, both of which would be useful for identifying mechanisms of injury and testing targets for therapy.

Metabolic Memory

The phenomenon of hyperglycemic memory presents a paradox: Patients in the DCCT with long-term exposure to a higher level of hyperglycemia remained more susceptible to complications, even with subsequent lower levels of hyperglycemia. In contrast, lower levels of hyperglycemia made patients more resistant to damage from subsequent higher levels. How can a finite period of different degrees of hyperglycemia result in different susceptibilities to complications? The discovery of the molecular and cellular basis of both types of metabolic memory is urgently needed so that solutions can be designed to prevent or reverse the damaging "memory" of high hyperglycemia, and to mimic or induce the protective "memory" of lower levels of hyperglycemia. Unlike the pathogenesis of diabetic complications, the molecular mechanisms underlying metabolic memory are virtually unexplored.

Research Objective—Discover the Molecular Mechanisms of Metabolic Memory:

- *Study epigenetic factors involved in metabolic memory.*

The Director of the National Human Genome Research Institute recently noted that there is an emerging recognition that scientists must move beyond their longstanding focus on the inherited "spelling" of people's DNA code and the occasional mutation or outright "misspelling." He noted that epigenetic changes do not alter genetic spellings, but may account for many cases of cancer and other diseases.

Human cells have tens of thousands of genes, each with its own job, such as producing energy or overseeing cell division. But only certain genes are active at any given time or in a given cell type, while the rest are appropriately dormant—a grand orchestration that adds up to a smooth-running life. This orchestration is determined by environmentally induced changes in molecules that coat the DNA. It has long been known that even identical twins have minor physical variations and differences in characteristics, such as susceptibility to disease. Recently, two dominant epigenetic changes were studied in identical twins: (1) DNA methylation, in which enzymes inside a cell attach a minuscule molecular decoration to a gene, deactivating that gene; and (2) histone acetylation, in which a dormant gene is made active again by the attached chemical group. These altered genetic settings can last a lifetime, and could be important for diabetic complications, if hyperglycemia can lead to these permanent genetic alternations. For example, a period of hyperglycemia could irreversibly turn off a gene that protects against diabetic complications. The ability of hyperglycemia to elicit epigenetic changes may be associated with different stages of development. Therefore, it could lead to treatment strategies that would promote intensive therapies during critical windows of development.

- *Investigate the role of mitochondria in metabolic memory.*

Much progress has been made in understanding the complex biology of mitochondria, which are the major source of hyperglycemia-induced ROS. Scientists now recognize that mitochondria are not all the same, but rather have important functional differences. Furthermore, mitochondria are not static structures in the cell. Rather, they continuously fuse to form larger organelles or pull apart to form smaller organelles. The processes underlying these changes are beginning to be understood, but aberrations induced by diabetes and different degrees of hyperglycemia are important new areas that will likely yield new insights into hyperglycemic memory.

- *Understand the regulation of the antioxidant response element.*

Perhaps not surprisingly, cells have their own protective antioxidant machinery. In a nematode model organism, cells responded to oxidative stress by activating a previously sequestered transcription factor (called Nrf2 in humans), which controls the expression of a diverse set of genes involved in decreasing ROS in the cell. An important research focus is the identification and regulation of proteins in this pathway, including proteins that bind to a special promoter element called the antioxidant response element (ARE) after activation by ROS. Such research will be critical to advancing understanding of hyperglycemic memory.

Genetic Factors

As with all complex diseases, the occurrence and progression of diabetic complications vary markedly among patients. Some patients have type 1 diabetes for over 50 years with minimal complications, while others manifest severe disease or death within 15 years after diagnosis. The control of blood glucose, as well as blood pressure and blood lipid profiles, are important factors in predicting the risk of complications, but they only partially explain the risk of complications for an individual patient. Therefore, genetic factors have been investigated for their influence on the risk of developing complications. An understanding of the genes involved in the susceptibility to or protection from diabetic complications can lead to both a better understanding of the pathophysiologic mechanisms, as well as new biomarkers and molecular targets for drug development.

Research Objective—Identify Genes Conferring Susceptibility and Resistance to Diabetic Complications:

- *Determine the genes that increase susceptibility to diabetic complications.*

The DCCT and other independent studies of patients with type 1 diabetes and their close relatives have shown that the incidence of nephropathy (and to a lesser extent retinopathy and neuropathy) in one sibling increases the risk that other siblings will develop the same complication. These studies provide evidence for a genetic component to the risk of developing complications. Possible candidate susceptibility genes have been selected that encode proteins thought to play a role in several known mechanisms of diabetic complications. Using this candidate gene approach, researchers have made numerous associations between genetic polymorphisms and the risk of diabetic complications.

Current strategies to meet this objective involve the following three ongoing research consortia, which are addressing the genetic factors that either predispose patients with diabetes to or protect them from developing complications: (1) Genetics of Kidneys in Diabetes Study (GoKinD); (2) Family Investigation of Nephropathy and Diabetes (FIND); and (3) Epidemiology of Diabetes Interventions and Complications Study (EDIC). These consortia have collected a large number of samples from patients and families with and without diabetic complications, which they plan to release to interested investigators. One important strategy to validate findings from the human studies will be through the use of animal models. Candidate genes could be tested for their effects in animals through the use of the Animal Models of Diabetic Complications Consortium (AMDCC) and the Mouse Metabolic Phenotyping Centers (MMPC).

- *Discover genetic modifiers for diabetic complications.*

As genes are identified that impact susceptibility to diabetic complications, a new area of research has emerged that will make it possible to identify genetic modifiers of the clinical manifestation of complications. With the completion of the genetic map known as the International HapMap Project, and new high-throughput genotyping technologies, this promising area of research holds great potential for understanding genetic determinants of the varying clinical severity of diabetic complications. These modifying genes are genetic variants that are distinct from disease susceptibility genes and that modify the phenotypic and clinical expression of the disease genes. Studies show that genetic modifiers can be "tipping point" genes. This term means that one gene changes the whole phenotype in an all-or-nothing fashion, much like switching a power switch "on" or "off." This paradigm contrasts with the incremental effects seen with changes in a large number of nonmodifier genes. Many examples of modifier genes are known in humans and model organisms. In fact, the most general lesson learned from experiments with genetically engineered mice may be the profound influence of genetic background on the phenotypic consequences of the engineered variant. Early studies suggest that genetic variants with modifier effects are probably also common and diverse in humans.

Because complications are likely to result not only from hyperglycemia, but also from a susceptibility to later pathophysiologic steps, such as inflammation or aberrant angiogenesis, a number of modifier genes may be relevant to diabetic complications. Discovery of these modifier genes will require integrated studies of different strains of mice, and comparisons of their genetic similarities and differences.

Accomplishing this research objective will also require careful characterization of patients with type 1 diabetes who have increased susceptibility or resistance to diabetic complications.

Animal Models

Animal models that mimic the human development of diabetic complications are desperately needed for research on mechanisms and for drug development. An essential step in developing new therapeutics is to test the efficacy of these agents in animals. Despite the remarkable genetic and physiologic similarities between humans and animal models in both health and disease, many other properties are unique to humans. This limitation means that animal models, while enormously informative, are only indirectly relevant to human disease. One of the reasons is that, in comparisons of mouse versus human genes, "similar" is not "the same." This distinction means that even subtle differences in gene sequence can lead to functionally important differences in suppression or enhancement of the phenotype by non-conserved amino acids, and responses in mice to potential human therapeutic agents may be very misleading.

Research Objective—Develop More Human-like Animal Models of Diabetic Complications:

- *Develop human-like mouse models for diabetic complications.*

A major opportunity in this field stems from cancer research, where mouse models with greater fidelity to human disease are made by substituting critical human genes for the mouse equivalent. Fortunately, significant advances have been made in the genetic modification of animals, so studies to replace rodent genes with human or human-like genes are feasible.

Engineered animal models of diabetic complications with human versions of genes will have more direct relevance to questions about complications pathogenesis and, equally important, will have a much greater accuracy in predicting which novel therapies will most likely work in humans. The AMDCC has already produced several new mouse models of diabetic heart, vascular, and kidney disease, and is organized to create better mouse models with relevant human and human-like genes. The MMPCs provide standardized, high-quality metabolic and physiologic phenotyping services for mouse models of diabetic complications.

- *Utilize large animal models of diabetic complications.*

In addition to mice engineered for relevance to human disease, large animal models that more closely resemble human physiology and disease development will also be needed to accelerate the process of "bench-to-bedside" research in the search for new, effective therapies for diabetic complications. For example, inducing diabetes in pigs with the drug streptozotocin provides a model of atherosclerosis that is relevant to this complication of human type 1 diabetes. Validation in such large animal models of potential therapies found effective in mouse models would greatly help to narrow the field of compounds for treating complications that are most likely to succeed in human trials.

Furthermore, large animals may be useful models of complications for which mice are physically too small. For example, reduced nerve blood flow is currently one of the most popular hypotheses for the generation of nerve damage in diabetes, but it has not yet been possible to measure this in mice, whereas larger rodents are widely used for such studies.

Biomarkers and Surrogate Endpoints To Facilitate Clinical Trials

The multi-organ damage caused by type 1 diabetes progresses silently for many years before presenting clinically with the signs or symptoms of disease. It then takes many more years before the occurrence of a well-defined event, such as a heart attack or kidney failure. Therefore, detection of early damage to cells and tissue by newly discovered biomarkers is critical for risk stratification of patients. In addition, as tissue damage progresses, the pathophysiologic mechanisms involved in progression are likely to include many more complex elements than are involved in the early initiation phase. This complexity makes therapeutic development more difficult and reversibility of the damage less likely. However, exciting results from islet transplantation trials have provided evidence that some complications can be reversed after many years of normal glucose levels.

Biomarkers include the results of a variety of procedures, including laboratory tests, biopsies, clinical testing, and diagnostic images. Development and validation of biomarkers occur over several phases, from discovery of molecular targets or development of new technologies, to testing with patients and controls, to validating results in clinical trials. Examples currently in clinical use include excretion of small amounts of protein in the urine as a biomarker for diabetic kidney disease, exercise echocardiograms as a clinical test for heart disease, and intravascular ultrasound as a diagnostic imaging technique for evaluating atherosclerosis.

Research Objective—Identify Biomarkers or a Combination of Biomarkers for Earlier Detection of Cell and Tissue Damage:

- *Validate newly developed biomarkers.*

Newly developed biomarkers that need further evaluation include: (1) measurement of intraepidermal nerve fiber density in small skin biopsies as a biomarker of diabetic peripheral neuropathy; and (2) images obtained from noninvasive magnetic resonance imaging (MRI) techniques as a biomarker of diabetic coronary artery disease. Additionally, the development of functional and qualitative assays of endothelial progenitor cells may be useful as biomarkers for cardiovascular risk.

- *Discover specific molecular targets and innovative technologies for early biomarker development.*

Research is urgently needed to optimize measurements of known molecular pathologies, such as ROS production and RAGE expression *in vivo*. Discovery of new biomarkers will encompass signature patterns of gene expression (genomics) or protein expression (proteomics). In addition, integration of these approaches with novel, noninvasive imaging techniques holds particular promise for evaluating metabolic and pathologic changes over time. For example, magnetofluorescent, multimodal nanoparticles have been successfully targeted to activated vascular endothelial cells *in vivo* using phage display-derived peptide sequences.

Collaborations among investigators having expertise in complex imaging technologies with investigators having expertise in the molecular cell biology of diabetic complications are likely to produce major advances in this field. These advances will then need to be validated in large clinical trials.

Research Objective—Validate Surrogate Endpoints for Assessing the Progression of Complications in Clinical Trials:

- *Develop surrogate endpoints for clinical trials in diabetic complications.*

At present, the development of therapeutics for diabetic complications is severely constrained because the slow progression rate of complications requires clinical trials of long duration in order to detect changes in outcomes. Surrogate endpoints are biomarkers that are strongly associated with and predictive of disease outcomes. Valid surrogate endpoints can measure the potential of new therapeutics, as well as be used to provide a strong scientific rationale for longer clinical trials and their prioritization. They would decrease the risk and improve the planning of clinical trials, and thereby encourage development of therapeutics for the complications of diabetes. Developing surrogate endpoints for diabetic complications is an important goal for all the groups involved in drug development, and collaborations among these groups will speed validation and acceptance of new endpoints.

Therapies To Improve Patient Health

Hyperglycemia and the other metabolic effects of type 1 diabetes cause cell and tissue changes that have the potential to be prevented or reversed by treatment. Beyond the vascular diabetes complications, there are psychosocial morbidities associated with the chronic disease that impair quality of life and limit the ability to optimally manage diabetes. Thus, researchers should pursue an array of strategies to prevent or reverse diabetic complications and improve patients' quality of life. Therapeutic approaches range from finding and testing agents to selectively modify molecular targets responsible for diabetic complications, to combining behavioral and technological approaches to improve patient management and glycemic control.

Research Objective—Identify Therapeutics That Prevent or Reverse the Development and Progression of Diabetic Complications:

- *Use high-throughput screening of molecular libraries to find new therapeutics for diabetic complications.*

The great success of new drug development over the past 40 years was based on using naturally occurring molecules as leads to design close analogues and derivatives. Classical bioassays and biochemistry were used to select compounds that competed with the native molecule for the same active site. Over the past decade, however, this model has been supplanted in large part by a new strategy. This new strategy involves the automated synthesis of large numbers of molecules (called a library) through a process called combinatorial chemistry, followed by screening in rapid biological assays in a process called high-throughput screening (HTS).
A demonstration project that is screening a library of FDA-approved drugs in assays for diabetic complications is currently under way. In addition, it will be essential to outline and have available critical follow-up mechanisms to assist investigators in developing lead candidate molecules in the process that leads to clinical trials.

- *Improve the high-throughput assays for diabetic complications.*

HTS has been tried for diabetic complications, but the

general consensus is that the HTS assays used were a poor simulation of the *in vivo* processes involved in diabetic complications. To find effective new drugs, it is essential to create and optimize cell- and simple organism-based models of the processes involved in determining the initiation, progression, and regression of diabetic complications. These assays can also be used to test libraries of existing drugs to determine their effectiveness in pathways relevant to diabetic complications. Several of the major research advances described earlier in this chapter have identified specific molecular targets or pathways that need to be pursued as targets for drug development. Other areas still need targets to be identified. One important example is the search for a molecular target that determines the regenerative capacity of diabetic blood vessels and other structures damaged by diabetic complications.

- *Apply the latest advances in drug development technology to diabetic complications.*

Through use of the combinatorial chemistry-HTS approach alone, fewer than expected viable drug candidates have reached the stage of clinical trials. More recently, a complementary strategy to HTS has emerged. This process uses computer-based virtual screening, multidimensional compound property optimization, and *de novo* design of drug-like molecules, which make it possible to identify not just active compounds, but compounds with high potential for optimization into drug-like lead series. While later stages of drug development require the substantial financial resources of pharmaceutical and biotechnology companies, the highly innovative NIH Molecular Libraries Initiative (MLI), a component of the NIH Roadmap for Medical Research, complements the private sector drug discovery effort by creating and screening a broader range of compounds, and assaying their effects on a broader range of targets. A significant expansion of the MLI into the area of diabetic complications could link innovative and creative academic researchers with the tools and expertise of small molecule discovery and development. This approach would decrease the time required for drug development and greatly improve prospects for clinical success. Mechanisms to enhance interaction between diabetes investigators and scientists leading the NIH Roadmap initiatives would also help facilitate more rapid development of candidate molecules for testing in type 1 diabetes.

- *Encourage the translation to human application of promising new therapies.*

There are a number of critical steps in the translational process for developing new therapeutic agents. Critical for fulfillment of the promise of HTS is that all promising leads undergo careful testing using the best available animal models of diabetes complications. These tests need to be conducted rigorously and in parallel with validated and consistent outcome measures. The Diabetic Retinopathy Clinical Research Network (DRCR.net) has been a successful model using private and academic clinical practices to test new therapies for diabetic retinopathy. Similar clinical networks for complications, such as nephropathy, neuropathy, wound healing, or cardiovascular disease, may help propel the translation of therapies to the clinic. Animal studies are a critical prelude to testing the most promising new therapeutic agents in human patients. Given the long timeframe necessary to definitively validate new therapeutic agents in humans, strategies to assess the promise of new agents in early phases of clinical testing are critical. This early clinical testing will likely require the use of a panel of existing and new markers for human disease.

Research Objective—Mitigate Psychosocial Complications and Comorbidities of Diabetes To Improve Quality of Life:

- *Clarify the bidirectional influences of depression as a complication and potentially modifiable risk factor for type 1 diabetes complications.*

Depression commonly occurs in patients with type 1 diabetes. In addition to depression in patients with type 1 diabetes, high rates of depression are observed in their parents and may have serious consequences for family quality of life and diabetes management. Depression in patients with type 1 diabetes is associated with hyperglycemia, increased insulin resistance, and increased risk of complications, particularly coronary heart disease. These observations are not entirely explained by the adverse effects of depression on behavior (e.g., decreased adherence to medical therapy, smoking, inactivity, obesity) and may reflect other psychophysiologic factors. Research is needed to determine whether such factors exist, and how they may adversely affect the course of diabetes. Conventional antidepressant treatments are effective in the presence of diabetes, with depression improvement leading to significant reductions in HbA1c levels. Some antidepressant drugs may have beneficial effects on diabetic neuropathy. Clinical studies could compare pharmacologic and nonpharmacologic management approaches, singly and together, in primary and secondary prevention of depression in type 1 diabetes, to evaluate the effects of antidepression treatments on diabetic complication outcomes.

Research Objective—Combine New Technology for Diabetes Management with Behavioral and Translational Research:

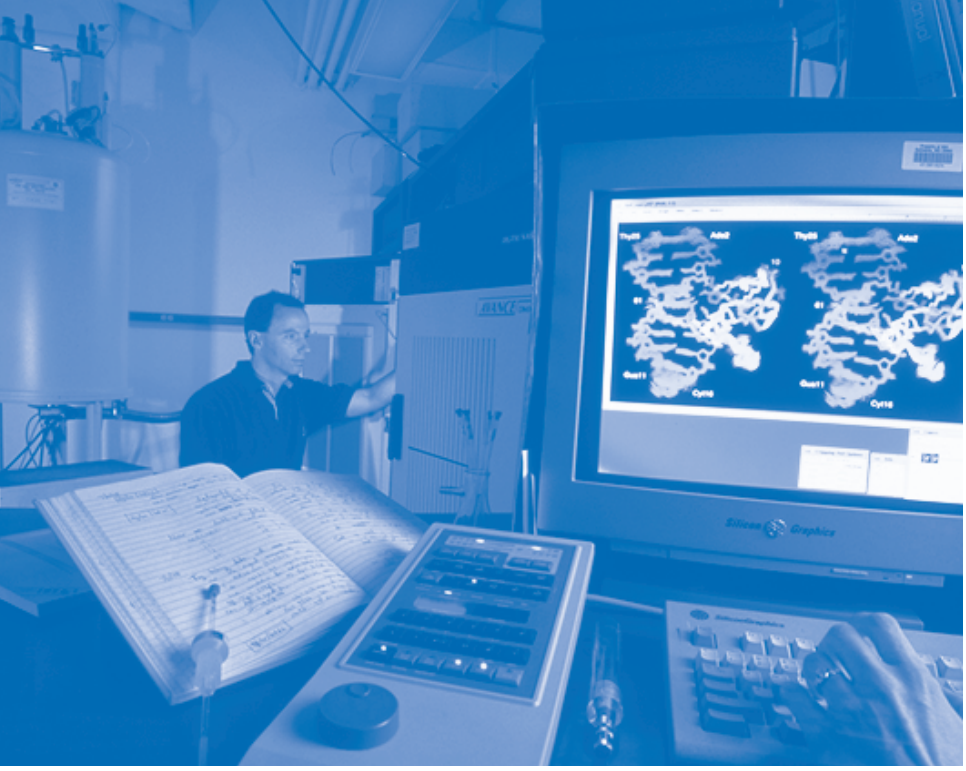
- *Design family-based interventions to improve patient management of diabetes.*

The diagnosis of type 1 diabetes can plunge a family into a long-term crisis mode with parents overwhelmed by the associated burdens and fears. Diabetes-related family conflict is a key factor limiting diabetes control. Children and families who show psychosocial adjustment difficulties early after diagnosis are at risk for poor long-term behavioral adaptation to diabetes. Research is necessary to develop behavioral approaches that improve family function and, ultimately, metabolic control in the patient. This will involve studying how patients and their families perceive information, adapt to new technology, and think about risk, in order to learn what motivates them to initiate and maintain behavioral change.

- *Identify strategies to improve adherence to therapy in adolescents and young adults with type 1 diabetes.*

The concept of metabolic memory (described previously) argues for promoting good glucose control as early as possible after diagnosis. However, it has been documented that adolescent years are characterized by lower adherence and elevated HbA1c levels. There is strong evidence that insulin resistance of puberty, the changing nature of the parent-child relationship, and the normal developmental tasks of autonomy, identity formation, and peer affiliation all contribute to suboptimal diabetic control in adolescence. Furthermore, empirical evidence has documented that loss to medical follow-up is a strong predictor of later complications, and the young adult period (18-30 years of age) is a vulnerable time for erratic medical care. Controlled studies have identified a range of strategies for improving glycemic control during the early adolescent years—for example, reducing family conflict over diabetes management, improving parental support for diabetes management, and negotiating realistic expectations for adolescent behavior and blood glucose levels. However, investigations are needed to determine the most effective ways to translate these strategies into routine pediatric care for adolescents with type 1 diabetes and their families.

Figure Legend: New technologies are helping to accelerate research on type 1 diabetes. *(Photo credit: Richard Nowitz for NIDDK.)*



GOAL VI:

ATTRACT NEW TALENT AND APPLY NEW TECHNOLOGIES TO RESEARCH ON TYPE 1 DIABETES

Recent Scientific Advances

Novel Imaging Technologies To Monitor Type 1 Diabetes Disease Progression and Islet Transplantation

Systems Biology Approaches Reveal Identity of Genes Involved in Pathophysiology of Diabetes

miRNA Involved in Regulation of Insulin Secretion

Brain Imaging in Hypoglycemia Unawareness

Engineering an Endless Source of Beta Cells for Therapy

Research Objectives and Strategies To Achieve Goals

Engaging Talented Scientists

- Recruit Expertise from Diverse Fields
- Design Incentives That Reward Research Innovation
- Train New Scientists in Clinical Type 1 Diabetes Research

Development and Application of New Technologies

- Develop Noninvasive Imaging Technologies To Monitor Type 1 Diabetes
- Promote Application of Advances in Bioengineering to Type 1 Diabetes
- Foster Application of Gene Delivery and Gene Silencing Technology To Develop New Therapies for Type 1 Diabetes and Its Complications
- Apply New and Emerging Technologies in Functional Genomics, Proteomics, and Metabolomics to Type 1 Diabetes Research
- Improve the Power of Diabetes Research by Utilizing Computational Biology and Bioinformatics
- Apply New Technology to the Development of Improved Animal Models for the Study of Type 1 Diabetes

STRATEGIES TO ATTRACT NEW TALENT AND APPLY NEW TECHNOLOGIES TO RESEARCH

INTRODUCTION AND BACKGROUND

In the last 100 years, doctors and scientists have made remarkable progress in the understanding and management of type 1 diabetes. As a result, people with the disease are now living longer and healthier lives. As the quest for a cure continues, progress will increasingly require collaborations among clinical and basic scientists with diverse skills and expertise. To understand the complicated interplay of hereditary and environmental factors that cause the disease and the progression of its complications (Goals I and V), geneticists and epidemiologists are beginning to collaborate with biostatisticians and informational biologists to generate computer models that will allow them to understand and test these complex interactions in the biological system. Preventing and reversing the chain of events in autoimmunity and achieving immune tolerance for organ transplants (Goals II and III) will require cooperation between immunologists and clinicians, as well as the biotechnology industry that develops and tests therapeutic agents. Similarly, knowledge of the basic biology of the insulin-producing pancreatic beta cell (Goal III) has expanded because of efforts to recruit cell and developmental biologists, who may have been focused on other systems and diseases, to the study of diabetes. These talented scientists are now directing their skills toward understanding how the beta cell develops. The research challenge of hypoglycemia unawareness (Goal IV) will require recruiting more neurobiologists and endocrinologists to understand the brain circuitry and body interactions. Continuing the progress that has been made in early detection and slowing progression of diabetes complications (Goal V) will involve not only experts in heart, nerve, kidney and eye disease, but also experts in proteomics, imaging, and other skills needed to develop biomarkers and surrogate endpoints that can speed translation of new therapeutic concepts from the bench to the bedside. For each of the Strategic Plan's scientific goals, a crucial prerequisite is to recruit and retain talented scientists and to foster collaborations, in order to propel further scientific advances.

With the recruitment of appropriate talent to the study of type 1 diabetes, cutting-edge technologies can be applied or developed for use in basic and clinical research. Certain technology themes cut across all the research goals and objectives outlined in this Strategic Plan. For example, the application of biophysical tools, such as labeled tracers, has opened the door for the use of new and improved methods of noninvasive imaging in both patients and animal models. These techniques will allow clinicians to assess the onset and progress of disease and the success of various therapeutic interventions. In recent years, biomedical research has witnessed an explosion of innovative tools that have paved the way for new fields of research, such as proteomics, functional genomics, metabolomics, bioinformatics, gene therapy, and gene silencing (siRNA). Scientists are rapidly applying these new technologies to type 1 diabetes research. However, it would also be beneficial to design new technologies in the context of type 1 diabetes research, so as to address the unique challenges of this disease. Additionally, new technologies may facilitate identification and validation of improved biomarkers for disease progression. Such biomarkers would make it less expensive and more efficient to conduct clinical trials and would thereby encourage industry investment in new therapies, from which patients might benefit more quickly.

RECENT SCIENTIFIC ADVANCES

Novel Imaging Technologies To Monitor Type 1 Diabetes Disease Progression and Islet Transplantation: Type 1 diabetes is usually diagnosed very late in disease progression, when most of the insulin-producing beta cells of the pancreas have already been destroyed. The development of a "toolbox" of imaging technologies capable of detecting the first signs of beta cell destruction would help to monitor therapy against immune attack or look for possible regeneration of beta cells. The first steps have been taken—scientists have recently developed a new, noninvasive imaging technology to monitor infiltration of inflammatory cells into the pancreas in an animal model of type 1 diabetes. This approach is now being tested in people. If successful, it could dramatically improve the ability of researchers to perform type 1 diabetes clinical trials.

Another important advance is the successful labeling of isolated human islets, mouse islets, and mouse T cells with nontoxic imaging probes that can be detected with magnetic resonance imaging (MRI), fluorescence, or nuclear imaging. The islets have been imaged quantitatively over time after implantation in the liver or under the kidney capsule in mice. T cells have been seen as they infiltrate the pancreas of a non-obese diabetic (NOD) mouse. Although such molecular imaging approaches are still very new, their application is now being introduced into human patients. It is hoped that they will soon be used in studies of type 1 diabetes.

Scientists are also exploring the use of positron emission tomography (PET) imaging to see radiolabeled ligands targeted to the pancreas. If such an approach proves successful, it would allow physicians to estimate the number or mass of a patient's own endogenous beta cells and to monitor the fate of transplanted islets.

Systems Biology Approaches Reveal Identity of Genes Involved in Pathophysiology of Diabetes:

Some diseases are caused by changes in a single gene that lead to a defective or missing protein, but complex diseases may involve subtle changes in the concentrations of a whole network of proteins working in concert. These changes can often be detected as a function of the concentration of the mRNA molecules that arise from DNA and code for proteins. The DNA microarray is a powerful tool that permits geneticists to simultaneously monitor the changes in gene expression (mRNA) of an entire genome to compare healthy and diseased tissues. Computational scientists are now working with biologists to develop adequate tools to analyze the vast amounts of data produced in each experiment, in order to more fully understand its value. Bioinformaticists recently introduced just such an analytical strategy that enabled them to compare gene expression in muscle biopsies from diabetic and non-diabetic individuals. Their analysis allowed them to identify a set of genes for which the expression is coordinately decreased in diabetic muscle. This group of genes carries out energy production in mitochondria. The affected mitochondrial protein genes are controlled by two transcription factors. Therefore, the few genes that code for these special transcription factor proteins regulate the expression of many other genes. Rare forms of monogenic diabetes, such as Maturity Onset Diabetes of the Young (MODY), provide another example of the key role of selected transcription factors in the pathogenesis of diabetes. These disorders originate from mutations in key transcription factors affecting entire networks of genes that regulate function in organs, such as the liver and pancreas. Using antibodies raised against a transcription factor known to be involved in pancreas development and liver metabolism, scientists can isolate all of the regions of the genome that bind to the transcription factor (chromatin immunoprecipitation or ChIP). The sequence of the DNA regions binding to the transcription factor of interest can then be identified using either large-scale sequencing strategies (Serial Analysis of Chromatin Occupancy or SACO) or hybridization on promoter microarrays containing the promoter regions of all known genes (ChIP-on-chip). These genome-wide analyses can result in fast, complex, and accurate modeling of transcriptional regulatory networks involved in the control of energy homeostasis, pancreatic beta cell function, or pancreatic islet mass.

miRNA Involved in Regulation of Insulin Secretion:

A novel class of natural molecules that controls translation of specific proteins was recently discovered and characterized. These are microRNAs (miRNA)—small single stranded chains of nucleic acid derived from non-coding portions of the genome. Building on this new discovery, researchers have recently found an miRNA in mice that suppresses insulin secretion. Using an miRNA-related technology called RNA interference (see sidebar on page 86), the researchers were able to mimic the effects of the miRNA to regulate insulin secretion. Furthermore, *in vivo* studies of chemically engineered oligonucleotide inhibitors of miRNA, called antagomirs, indicate that a single miRNA is likely to have not one but many gene targets. Therefore, antagomirs are powerful tools that can silence *in vivo* miRNA-controlled regulatory pathways and could become a therapeutic strategy for pathologies in which miRNAs participate in disease etiology.

Brain Imaging in Hypoglycemia Unawareness:

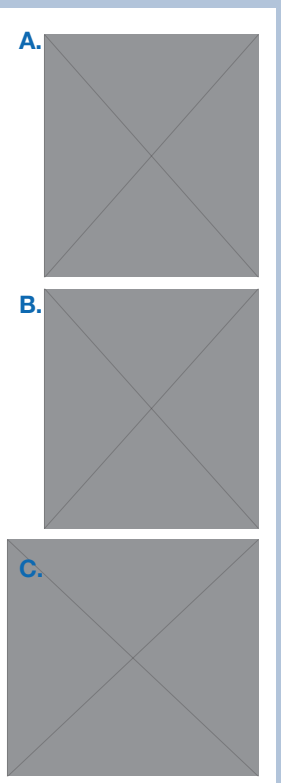
The brain is dependent on glucose for fuel, and it also contains glucose-responsive neurons in specialized regions that sense blood glucose concentration and then coordinate the hormonal, neurological, and behavioral responses that rescue a person when his or her blood glucose levels sink too low. Diabetes can be accompanied by a failure of the brain to both recognize hypoglycemia and generate signals to prevent blood glucose from falling to a dangerously low level. This hypoglycemia unawareness often develops after repeated episodes of hypoglycemia and is very frightening to diabetes patients who may lose consciousness without warning. Now, scientists are beginning to understand how the brain loses its ability to respond and warn of impending hypoglycemia. New imaging technologies have revealed differences in glucose use in different areas of the brain. Imaging has also shown that the brain increases blood flow when blood glucose falls and that its cells store glucose, which can be used when it cannot get enough from the blood. Combined, these mechanisms may help maintain glucose availability to the brain, and

thus cognitive function, when the body experiences hypoglycemia. Most people with diabetes have the same brain glucose concentrations as healthy people, but brain glucose is elevated in those who develop hypoglycemia unawareness; thus, glucose levels may stay higher in their brain cells even as blood glucose levels fall, masking the normal warning to respond to low blood glucose and, therefore, failing to prevent it. Considerable progress remains to be made in this area before achieving a true understanding of how the brain reacts to glucose concentrations and what goes awry in hypoglycemia unawareness in diabetes. However, the new imaging technologies of MRI and PET have opened the window on the brain and its metabolism.

Engineering an Endless Source of Beta Cells for Therapy: The limited supply of human islets available for transplantation dramatically reduces patient access to this potentially life-enhancing therapy. In laboratory studies, efforts to produce an increased quantity of glucose-regulated, insulin-producing cells for transplant have employed cell culture, tissue engineering, and gene therapy technologies to promote beta cell development from adult or embryonic stem cells. In addition, mature cells from tissues such as liver, spleen, intestine, or pancreas, or from cultured cell lines, have been tested for their ability to serve as donors in cell-based therapies. To enhance their therapeutic potential, these cells have been incubated with growth- and transcription-activating factors or hormones, co-cultured with additional cell types, or manipulated to express genes that code for proteins found in the beta cell. Insulin-secreting beta cell-like immortalized cell lines have been created by careful selection for beta cell specific traits. These efforts have yielded cells that, in some cases, are able to reverse diabetes in animal models, but have not yet been fully successful in reproducing the exquisite sensitivity to small changes in glucose levels that is characteristic of mature beta cells. This research has also provided considerable insight into the characteristics that make up a beta cell and a foundation for further progress toward improving the supply of beta cells or islets.

Potential Therapeutic Applications of RNA Interference (RNAi) in Type 1 Diabetes

The recent discovery of the natural molecules known as microRNA (miRNA) has challenged the prevailing scientific thinking regarding the role of ribonucleic acid (RNA) in gene regulation. The miRNA has much shorter chains of nucleic acids than messenger RNA (mRNA), which contains the coding sequences of proteins. These miRNAs can specifically silence the expression of a gene or a family of genes by blocking translation of the proteins they encode, and they are involved in the regulation of a wide variety of cellular functions, ranging from cell fate determination to suppression of glucose-induced insulin secretion in the beta cell. Mammalian genomes contain a large and diverse family of miRNAs; it is now believed that miRNAs, conserved across evolution from plants to animals, may affect one-third of all human gene expression. However, because scientists have spent decades focusing on protein-based mechanisms of gene regulation, the research tools for studying miRNA regulation are just now being developed.



Researchers are designing sequence-specific RNA interfering molecules (siRNAs) to silence expression of disease genes, such as genes involved in destroying beta cells in type 1 diabetes. The mechanism by which this technology works is: (A) A cell expresses a protein by transcribing genetic information stored in the DNA into messenger RNA (mRNA). The mRNA is then translated into a protein. (B) Researchers have identified a new type of RNA—microRNA—that comes from DNA, but does not code for proteins. Instead, the sequences of microRNA complement and bind to the sequences of specific "targeted" mRNA, thereby preventing the mRNA from being translated into a protein. (C) Small interfering RNA, or siRNA, is synthesized in the laboratory, but uses a similar mechanism to interrupt mRNA translation.

Researchers have learned how to manipulate the pathways used by miRNA by using synthetic double-stranded RNA molecules called small interfering RNA (siRNA). These are employed in RNA silencing or interference studies (RNAi) to suppress translation of the gene of interest and provide insight into its function. Successful *in vivo* delivery of siRNA has been demonstrated in a wide variety of organs, including the pancreas. This technology is quickly moving into the clinic, as several biotechnology companies have received FDA approval for conducting phase I clinical trials using RNAi-based therapies. In one such phase I trial, siRNA technology is used to target the vascular endothelial growth factor (VEGF) pathway to treat age-related macular degeneration; visual acuity improvement has already been reported in the treatment group. If successful, such an approach may be used for the treatment of diabetic retinopathy resulting from abnormal, VEGF-dependent angiogenesis in the retina. Other RNAi treatments in development are targeting specific immune and inflammatory responses. For example, a program using siRNA targeting key Th2 cytokines is entering a phase I trial for the treatment of asthma. RNAi-based therapeutic strategies targeting specific components of the immune response and/or the apoptotic pathways involved in beta cell destruction, combined with early detection of disease, could result in the restoration and maintenance of normal beta cell mass in patients with type 1 diabetes.

Engaging Talented Scientists

Pursuit of the full range of opportunities for prevention and improved therapy of type 1 diabetes and its complications requires a wide range of scientific expertise and the participation of investigators from diverse fields. These talented researchers must be recruited to the field, and promising new investigators must be trained in diabetes and enlisted in the research enterprise. High-yield, multidisciplinary approaches can only be fostered with appropriate new infrastructure. Finally, resources must be provided to promote an environment that values high-risk, high-impact projects. Tomorrow's ability to prevent and cure type 1 diabetes depends on the quality of today's research community and environment.

Research Objective—Recruit Expertise from Diverse Fields:

▸ *Encourage interdisciplinary collaborations.*

Type 1 diabetes affects many different organ systems and requires expertise in fields as diverse as genetics, neurobiology, immunology, biophysics, endocrinology, imaging, bioengineering, and biostatistics. The NIH has pioneered novel approaches to establish and empower new scientific teams for type 1 diabetes research.

Collaboration among scientists with complementary expertise has been forged via "innovative partnerships" (see sidebar) that encourage experts in type 1 diabetes research to recruit and work together with scientists from outside the field. In "bench-to-bedside" research partnerships, a team of clinical and basic scientists conducts collaborative research that, if successful, will bring basic research advances from the laboratory to a point where a potential new therapy can be tested in patients or in pre-clinical studies in animal models. A major obstacle to research is being alleviated by programs that provide scientists with access to collections of biological samples from well-characterized, consenting patients. The repositories that store and distribute such samples are an important resource that allows the creative research community to conduct mechanistic studies in virtual collaboration with the clinicians as they pursue patient-oriented research.

Scientific workshops bring together experts from different fields to stimulate discussions and cooperative endeavors in a particular field, and they have resulted in important new collaborations. For example, one such key workshop brought

Innovative Partnerships in Type 1 Diabetes: A Novel "Co-Principal Investigator" Support Mechanism

Because research on type 1 diabetes spans a broad range of scientific disciplines, propelling research progress requires a cadre of scientists with diverse research training and expertise. To attract new research talent to study type 1 diabetes and its complications, the NIH has supported an initiative on "Innovative Partnerships in Type 1 Diabetes Research." The overall objective of the initiative was to support collaborations between investigators who focus their research efforts on type 1 diabetes or its complications and investigators from other research areas with expertise relevant to type 1 diabetes. Type 1 diabetes researchers therefore acted as "talent scouts" by identifying and recruiting leading scientists with expertise relevant to the field of type 1 diabetes research. Using this mechanism, researchers with expertise in areas such as cell-based screening, imaging, genomics, and systems engineering are now pursuing research on type 1 diabetes.

The intent of the initiative was to encourage true partnerships in which two or more investigators with complementary expertise tackled a common problem. However, the standard policy at the NIH was to award a grant to only one principal investigator, while the partner was listed as a co-investigator—an arrangement that did not recognize both partners as being equal and thus posed a barrier to collaboration. Based on feedback received from the external scientific community, the NIH pioneered a novel solicitation so that both partners were named as co-equal principal investigators. This arrangement was first used under the *Special Statutory Funding Program for Type 1 Diabetes Research*. It provided an important incentive to collaboration, and attracted expertise from diverse fields. For example, one project brought together diabetes complications investigators with experts in angiogenesis (small blood vessel formation), thereby helping to move therapeutics currently used for cancer toward applications for diabetes complications. The new awards benefited both partners, who have now received equal recognition for their contributions to the research study. This recognition can be beneficial to investigators, who may be evaluated by their home institution in terms of the number of grant awards they have received. The "co-principal investigator" mechanism—first employed by the NIDDK with the "Innovative Partnerships in Type 1 Diabetes Research" initiative—is now being considered for broader implementation by the NIH as a whole, under the NIH Roadmap for Medical Research.

together clinical researchers from the landmark Diabetes Control and Complications Trial with cell biologists to explore the mechanisms underlying "metabolic memory" (see Goal V). Another workshop drew both neuroscientists and diabetes experts to address the problem of hypoglycemia unawareness that limits therapy for type 1 diabetes (see Goal IV). Attendees outlined collaborative strategies to elucidate the mechanisms underlying this condition and explored avenues to reverse it. A third workshop brought together proteomics experts and researchers in the fundamental and clinical aspects of diabetes research, yielding valuable suggestions for future efforts to develop much needed biomarkers and surrogate outcomes.

Research Objective—Design Incentives That Reward Research Innovation:

▸ *Promote high-risk, high-impact research.*

Scientists have identified certain barriers as critical bottlenecks that impede progress in type 1 diabetes. Particularly noteworthy is the need for biomarkers and surrogate endpoints to conduct clinical research to evaluate potential new therapeutics in small pilot trials. However, this type of discovery research is inherently risky, with no assurance of a positive outcome. Moreover, applicants find it difficult to obtain funding for high-risk research even if the work has potential for high, positive impact. Although not all high-risk research makes it to the clinic, investment in pioneering research may eventually stimulate the next major breakthrough. Therefore, it is imperative to provide incentives for talented scientists to undertake such research. These can take the form of limiting the requirements for preliminary data for pilot studies and of providing quick turn-around for continued or expanded funding when pilot studies meet defined milestones for achievement.

▸ *Create an environment conducive to innovation and collaboration.*

Although science has traditionally been fueled by competition, recent efforts focused on aspects of type 1 diabetes research have attempted to alter that paradigm and foster a community-oriented approach through the establishment of large research consortia, clinical trial networks, research centers, and team science. Such cooperation has enabled researchers to undertake large interdisciplinary projects that could not be pursued independently by any single investigator or small research group. Within a consortium, team members can share data, samples, protocols, research resources, and even cost-intensive patient recruitment in an efficient and effective way. The *Special Statutory Funding Program for Type 1 Diabetes Research* has not only created many effective consortia, but it has also recently provided support for infrastructure to promote cooperation among consortia. A consortia coordinating committee has been formed to resolve issues such as interoperability of databases and standardization of patient informed consent procedures. Furthermore, the NIH supports a website to announce the availability of research resources and funding opportunities to the research community (accessed at: www.T1Diabetes.nih.gov/investigator).

Research Objective—Train New Scientists in Clinical Type 1 Diabetes Research:

▸ *Attract and train new diabetes investigators.*

New scientists and engineers often bring energy and creativity to a field; future progress depends on the research training and mentoring of new students, postdoctoral fellows, and independent investigators. Currently, the *Special Funding Program* provides competitive institutional research career and training awards for pediatric endocrinologists involved in type 1 diabetes research. While development of pediatric endocrinologists as diabetes researchers was considered the highest priority for the limited resources available, this program could be productively expanded to promote research career development and training for investigators in other areas of importance to type 1 diabetes research. Programs that provide funding for exploratory projects could be very influential at the critical time when junior investigators are making the choices that will determine their long-term career paths.

Development and Application of New Technologies

The past decade has seen major advances in biotechnology with direct relevance to type 1 diabetes research. It is imperative to put cutting-edge technology into the hands of type 1 diabetes researchers and to foster future development of these tools in the context of their application to type 1 diabetes. Highlighted below are some of the most promising new technologies with important applications for type 1 diabetes.

Research Objective—Develop Noninvasive Imaging Technologies To Monitor Type 1 Diabetes:

▸ *Develop imaging for pancreatic beta cell mass, function, and inflammation.*

As discussed in the "Recent Scientific Advances" section of this chapter, there has been impressive progress in this area despite substantial challenges inherent in visualizing a tiny population of cells that reside deep inside the abdomen and

share many defining characteristics with neighboring cell types. Success in imaging will provide insights into the natural history of islets in diabetes; facilitate clinical trials to test therapies to slow or reverse beta cell loss; and allow physicians to monitor engraftment following islet transplantation. An islet imaging program funded by the NIH and JDRF has supported promising projects in animals and in humans looking at original surviving islets, as well as transplanted ones. Parallel projects are ongoing to provide the important reagents for imaging. These include the identification of unique cell surface proteins and production of monoclonal antibodies and other specific ligands that can be tagged for molecular imaging. The next steps are to create an environment in which various imaging approaches and reagents can be translated from the laboratory to clinical application in a well characterized patient population. This transition will require a balanced team of cutting-edge physicians, imaging experts, chemists, and biologists who have access to state-of-the-art equipment for imaging in both animal models and patients. Finally, entirely new approaches are being designed for imaging live tissues. These include:

- New optical tools that are more sensitive to small differences among tissues, can image more deeply into the body, or can take advantage of optical fibers and miniaturized detectors that can be introduced into the body in a mildly invasive manner;
- New x-ray imaging that can enable researchers to see soft tissues with very high resolution and reduced radiation exposure;
- New highly sensitive ultrasound and MRI contrast agents; and
- Enhanced resolution through powerful image reconstruction paradigms.

The diabetes research community must be positioned to take advantage of the best of these technologies as they appear, so that they can immediately be brought to bear on the challenging problem of imaging the pancreatic islet beta cell.

- *Develop brain imaging techniques to use in understanding hypoglycemia.*

How do minutes or hours of hypoglycemia affect structure, function, and metabolism of the brain? What are the short- and long-term consequences of multiple hypoglycemic episodes? What leads to hypoglycemia unawareness? To answer these questions, improved brain imaging with high spatial resolution is needed to elucidate the relationship between the specific neurons and their supporting cells involved in the detection and response to low blood sugar. It will likely be necessary to introduce artificial molecular imaging tags that bind to specific surface proteins in order to distinguish cell types from one another in glucose-sensing regions of the brain (see sidebar on page 90). The ability to visualize neural function is needed to understand hypoglycemia unawareness. There are several novel functional imaging technologies being used to study the brain, such as BOLD fMRI, arterial spin labeling techniques, diffusion tensor imaging, and magnetoencephalography. Because of the relatively small brain regions involved, the ability to study hypoglycemia may benefit from an investment in additional novel functional imaging tests. In addition to technology, experimental paradigms are needed that couple physiological responses measured by imaging with reliable measures of behavior in response to hypoglycemia.

Research Objective—Promote Application of Advances in Bioengineering to Type 1 Diabetes:

- *Develop novel drug delivery methods.*

Effective drug delivery depends on applying the proper dosage in the proper location over the proper time course, while overcoming issues of target specificity and drug degradation. Bioengineered drug-eluting polymers can be implanted to slowly release a drug over time directly at the site where it is needed, such as in the eye to treat diabetic retinopathy or directly in a foot ulcer. Cardiac stents can be coated with drugs that locally suppress immune reactions or prevent reocclusion of the vessel. Scientists are also embedding immune suppression drugs or compounds that promote angiogenesis or islet replication into materials used to encapsulate and protect islets for transplant.

- *Develop noninvasive glucose monitoring technologies.*

An artificial pancreas would require a continuous glucose sensor whose output could be used to regulate an insulin pump in a feedback loop. To achieve such a closed-loop system, glucose monitors are needed that are faster, more accurate, and easier to use. Flexible algorithms are needed to link the changes in blood or interstitial fluid glucose to insulin delivery. Although the artificial pancreas is not yet available, the first steps have been taken. The NIH supports basic sensor research in universities and industry, as well as clinical assessment of devices arising from these projects by independent academic investigators.

- *Integrate tissue engineering and regenerative medicine to develop tissues and organs to replace those destroyed by diabetes and its complications.*

Tissue engineering is an exciting emerging field in which biocompatible synthetic polymers, cells, and tissues, as

Imaging: An Inside Look

Seeing is believing. Imaging scientists are working to find ways to visualize the processes that lead to diabetes and how the body responds to therapy. These new tools will further a better understanding about how the disease starts and progresses. Imaging techniques will provide insights into why, how, and when diabetes occurs, as well as point to new ways for treating the disease.

The secret to imaging diabetes is the use of drug-like imaging agents that selectively "light up" the cells or biological processes involved in disease. For instance, the metals iron and gadolinium change the signal in magnetic resonance imaging (MRI). Compounds that contain these metals can be designed to home in specifically on the insulin-producing beta cells in the pancreas, thereby permitting them to be counted. Similar compounds have been used to light up the inflammation in the pancreas that accompanies the autoimmune destruction of the beta cells and causes type 1 diabetes. Other imaging agents mimic nutrients or hormones and, when taken up by cells, reveal clues to their function and metabolism. These types of agents are commonly labeled with minute levels of radioactivity and detected by positron emission tomography (PET). Thus, they might allow researchers to distinguish among active and distressed beta cells. Currently, considerable effort is focused on putting imaging labels on the isolated pancreatic islets used for transplantation into diabetic patients. This approach would enable doctors to actually watch the locations to which the transplanted tissues migrate once they are infused into patients and to determine their fate—that is, to know how many survive to produce insulin, find out whether they grow in their new environment, and see what happens to those that die. Imaging might also disclose the formation of new blood vessels and nerves around the islets, as well as reveal the importance of these processes for insulin secretion.

Scientists have learned to incorporate into mice a family of proteins that either emit light (such as the luciferase/luciferin system from the firefly) or fluoresce (such as green fluorescent protein). These constitute a very powerful set of imaging tools that are used in basic animal research. For instance, fluorescently labeled insulin can be tracked by the microscope to uncover defects in insulin secretion that might be involved in diabetes. It is hoped that these tools will help researchers identify and monitor a precursor cell that can become a new insulin-producing beta cell.

Imaging may one day help manage diabetes or identify patients prone to diabetic complications before they become clinically obvious. For instance, new glucose-sensitive imaging agents may make possible the continuous monitoring of plasma glucose without finger sticks. Such an advance would be enormously beneficial for patients. Therefore, scientists are working to bring emerging imaging tools to bear on all aspects of diabetes and its treatment.

well as gene manipulation technology, drugs, and natural biological molecules, are all brought to bear. Bioengineered tissues could improve therapy for diabetes complications: artificial skin for the repair of diabetic foot ulcers, heart muscle patches, and improved vascular access for dialysis. Glucose-responsive, insulin-secreting cells engineered from a readily available cell source may replace the human cadaver islets that are currently being used for transplantation and are in short supply. If a patient's own cells could be used as the precursor, this may alleviate the need for immunosuppressive drugs and greatly increase the number of patients who could be treated.

- *Apply nanomedicine to drug delivery, islet encapsulation, noninvasive imaging, and glucose-sensing technologies.*

The Office of Science and Technology Policy in the Executive Office of the President has launched a government-wide initiative to invest in the burgeoning field of nanotechnology. Nanotechnology is the manufacture, study, and use of molecules with unique properties when observed at a nanoscale level—larger than atoms, but much smaller than cells. This is a fertile field in which to engage engineers and scientists to work on the technologic challenges described previously with respect to type 1 diabetes research, including: imaging, tissue engineering, drug delivery, immunoprotective coatings, and development of an artificial pancreas.

Research Objective—Foster Application of Gene Delivery and Gene Silencing Technology To Develop New Therapies for Type 1 Diabetes and Its Complications:

- *Develop technology for gene delivery to cells and tissues that are therapeutic targets for type 1 diabetes.*

Gene therapy is an experimental approach to introduce into cells a gene that either replaces a mutated, disease-causing gene or provides a new cellular function. Complications, such as diabetic retinopathy, neuropathy, and wound healing, are potential targets for gene therapy. Instead of introducing vectors globally into the whole body, vectors could be applied directly to the affected site, which should reduce toxicity. Gene therapy has been used in an animal model to deliver growth factor genes to skin ulcers and has been successful in accelerating wound healing. Based on these studies, a human trial has been initiated using this approach to improve wound healing. Gene therapy applications are also being tested in animal models to deliver genes to the retina or to nerve cells to prevent cell damage. Gene therapy using viral vectors in a dog model has been successful in treating another disease of the retina, retinitis pigmentosa. Despite the fact that this

technology is still at a very early stage of development, these applications to diabetic complications are being actively pursued.

- *Create siRNA vectors for gene silencing in target tissues.*

As described in a sidebar, siRNA is a new technology that allows researchers to efficiently and rapidly reduce the level of expression of proteins in cells and tissues. Used as a research tool, *in vitro* and *in vivo*, siRNAs enable researchers to better understand the contribution of specific proteins to regulatory or disease pathways. For example, using siRNAs to specifically silence disease-causing genes in NOD mouse models will help geneticists dissect the particular contributions of these genes to the development of a diabetes phenotype. Similar techniques will allow immunologists to understand costimulatory pathways that control the balancing of immune cell function (e.g., effector and regulatory T cells). In addition to being used in basic science, siRNAs are being combined with gene therapy vectors to silence a variety of genes involved in disease onset and complications in patients.

Research Objective—Apply New and Emerging Technologies in Functional Genomics, Proteomics, and Metabolomics to Type 1 Diabetes Research:

- *Use "omics" technologies to identify interactions among genes, proteins, and metabolites in type 1 diabetes and its complications.*

Functional genomics is the new field of science that employs DNA microarrays to measure those genes that are active in a tissue under a given set of conditions and identifies clusters of genes that work together. Human and mouse "PancChip" microarrays were developed by the Beta Cell Biology Consortium (BCBC; see Goal III) and contain thousands of genes expressed specifically in the pancreas. This resource is critical for identifying the pathways involved in the development and function of the beta cell and is being distributed widely to investigators seeking to develop an unlimited supply of beta cells for cure of diabetes. The PancChip has significant importance for other diseases as well, such as pancreatic cancer.

The function of a gene is fulfilled through the proteins whose formation it directs. The complete set of proteins and their interactions, or proteome, provide further opportunities for systematic analysis and exploration. Proteomics involves the use of several novel integrated technologies to identify and quantitate proteins and study their interactions, modifications, and dynamics. Proteomic technologies have been successfully used for the identification of cancer biomarkers, elucidation of biochemical pathways, and pinpointing of novel drug targets. Metabolomics studies the small molecules, such as amino acids, carbohydrates, and lipids in the cells, tissues, and biofluids of an organism. The metabolome responds quickly to disease, diurnal and nutritional variation, and can be a very sensitive indicator of a person's current metabolic state. Large-scale approaches, such as proteomics, genomics, and metabolomics, are promising technologies for understanding the complex molecular mechanisms that underlie type 1 diabetes and its complications.

- *Utilize proteomic and metabolomic technologies to identify and validate surrogate markers that predict risk, rate of progression, or response to therapy for type 1 diabetes and its complications.*

A current major barrier in conducting type 1 diabetes clinical trials is the need for easily measured biomarkers that adequately predict disease risk and progression well before a measurable clinical outcome, such as diabetes onset, or a serious complication, such as a heart attack. "Omics" technologies will provide "fingerprints" or patterns of molecules that are diagnostic of disease and may be more powerful as clinical biomarkers than a single molecule, such as glucose or glycosylated hemoglobin. Many events that clinicians would like to monitor do not have such a single marker, such as the autoimmune process of type 1 diabetes or the early manifestations of diabetes complications. If these events could be identified, those individuals most likely to benefit from immunomodulation or who are at highest risk to develop complications could be intensively treated. Some effort in this direction has already begun. For example, investigators are now collaborating on proteomic projects to identify beta cell proteins that might give rise to the immune attack. Appropriate surrogate markers could dramatically enhance researchers' ability to conduct clinical trials, as well as shorten the duration of the trials.

Research Objective—Improve the Power of Diabetes Research by Utilizing Computational Biology and Bioinformatics:

- *Enhance type 1 diabetes research efforts by incorporating bioinformatics at the inception of the research effort.*

Bioinformatics is a newly emerging field that combines data storage, organization, and analysis. Bioinformatics has the power to correlate genetic, biochemical, cell function, demographic, and clinical data from disparate data sets from all over the world to create a comprehensive picture of disease. It has critical applications for analyzing complex data sets generated by clinical trials of immunomodulation and their associated mechanistic ancillary studies, or for analyzing genetic samples from thousands of patients with type 1 diabetes

and their families. In both instances, huge sets containing data as disparate as a patient's genotype and the immune cell complement in his or her blood will be analyzed together with descriptions of the clinical and biochemical manifestations of the disease. The efforts of bioinformatics experts will be critical to isolating in these populations of research patients the significant variables that either cause or protect against type 1 diabetes and its complications. It is expected that new hypotheses regarding the pathology of disease will be generated by searching for novel correlations within such data sets. Because of this, future data collections could benefit greatly from involving bioinformatics experts early in the study design. The ultimate goal would be to work toward interoperability, so that data stored in all these databases could be freely accessed and combined by the research community—efficiently and productively.

- *Apply computational biology to the complex systems in type 1 diabetes.*

The ability to organize complex data sets is only the first step for bioinformatics. As biology becomes increasingly sophisticated, computer models can save time and maximize the use of resources for analysis of complex systems. For example, insulin secretion involves networks of genes and metabolites interacting among all the cell types in the pancreatic islet, which, in turn, receives signals from the rest of the body in the form of hormones, nutrient levels, cells, molecules of the immune system, and nerve impulses. Computer models could help biologists predict how all of these signals are integrated to control blood glucose levels. Similarly, the development of an artificial pancreas (see Goal IV) will depend on developing algorithms that can use data on physical activity, diet, insulin administration, and glucose levels to calculate and effect fine-tuned insulin delivery.

- *Integrate information technology into type 1 diabetes self-care and medical management.*

Studies of the care of patients with a variety of chronic health problems demonstrate that information technology may improve health care access and quality for individuals with type 1 diabetes. Randomized trials have found that structured e-mail communication with health educators, interactive DVDs, and automated calls with nurse follow-up can all improve important physiologic endpoints, and in some cases may even improve survival. Future work is needed to demonstrate the effectiveness of a variety of communication tools for patients with type 1 diabetes, particularly as patients have access to unprecedented amounts of data generated by continuous glucose monitors. The goal is to maximize the impact of these technologies by empowering patients and providers to synthesize the data and use it to inform management. Research is needed to understand the cost-effectiveness of integrating computer supports into traditional treatment models and the extent to which these services should either supplement or substitute for face-to-face clinical visits.

Research Objective—Apply New Technology to the Development of Improved Animal Models for the Study of Type 1 Diabetes:

- *Develop models needed to identify cellular and molecular pathways influencing beta cell formation and function.*

Cell-based therapies designed to replace the beta cells lost due to immune-mediated destruction require a firm understanding of their developmental paths and fates, as well as a ready source of expanded cells for treatment. In pursuit of this goal, the BCBC is producing mice bearing fluorescent tags (the "Rainbow Mouse") that illuminate the developmental path followed by beta cells as they arise and begin to populate the pancreas. Investigators are extending this approach to produce animal models in which it is possible to identify the cellular source of newly arising beta cells, as well as the molecular pathways responsible for regulating beta cell growth and function in the adult pancreas. Through studies in these and other new animal model systems, it may be possible to identify novel molecular targets for therapeutics development in type 1 diabetes.

- *Develop animal systems with greater fidelity to human disease to enhance pre-clinical testing and biomarker development.*

In the Type 1 Diabetes-Rapid Access to Intervention Development (T1D-RAID) program, promising new drugs are being produced and tested in existing animal models for their ability to reduce type 1 diabetes autoimmunity or diabetic complications. These pre-clinical studies are being facilitated by the activities of the Animal Models of Diabetic Complications Consortium (AMDCC), which is developing new mouse models of diabetic complications. Particularly promising are new AMDCC models of diabetic cardiovascular disease, nephropathy, and neuropathy that will be of tremendous value for testing new drugs for these conditions. How closely these new models reproduce the human condition, and their ultimate utility as models for pre-clinical testing of new drugs, will be determined through the joint phenotyping efforts of the AMDCC and the Mouse Metabolic Phenotyping Centers (MMPC).

New mouse strains bearing HLA or other human disease susceptibility genes are providing systems to study the genetic components of human type 1 diabetes in mice. The value of newly discovered genes will be dramatically enhanced by the coming availability of new immunocompromised mouse models that allow for efficient reconstitution of the human immune system in mice. This important advance will, for the first time, allow in-depth mechanistic studies in mice of human autoimmunity, transplantation, and tolerance in the context of human genetic susceptibility loci and the human immune system.

While rodent models have been and continue to be a valuable tool for dissecting mechanisms of disease and for testing new drugs, studies in fish, pigs, and non-human primates are also providing valuable insights, including for the study of islet development and transplantation. As these models are validated and come into widespread use, they will allow for improved and more predictive tests of new therapies. Moreover, models in pigs and non-human primates may be particularly valuable tools for identifying new biomarkers of disease progression that are needed to improve type 1 diabetes clinical trial design and medical care.

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APPENDIX B: ACRONYMS AND ABBREVIATIONS

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Organizational Components

ADA	American Diabetes Association
CDC	Centers for Disease Control and Prevention
CMS	Centers for Medicare & Medicaid Services
HHS	Department of Health and Human Services
DMICC	Diabetes Mellitus Interagency Coordinating Committee
FDA	Food and Drug Administration
JDRF	Juvenile Diabetes Research Foundation International
NCRR	National Center for Research Resources
NEI	National Eye Institute
NHGRI	National Human Genome Research Institute
NHLBI	National Heart, Lung, and Blood Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIBIB	National Institute of Biomedical Imaging and Bioengineering
NICHD	National Institute of Child Health and Human Development
NIDA	National Institute on Drug Abuse
NIDCR	National Institute of Dental and Craniofacial Research
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke
NINR	National Institute of Nursing Research
NLM	National Library of Medicine

Research Programs

AMDCC	Animal Models of Diabetic Complications Consortium
BCBC	Beta Cell Biology Consortium
CIT	Clinical Islet Transplantation Consortium
CITR	Collaborative Islet Transplant Registry
DCCT	Diabetes Control and Complications Trial
DirecNet	Diabetes Research in Children Network
DPT-1	Diabetes Prevention Trial-Type 1
DRCR.net	Diabetic Retinopathy Clinical Research Network
EDIC	Epidemiology of Diabetes Interventions and Complications
FIND	Family Investigation of Nephropathy and Diabetes
GoKinD	Genetics of Kidneys in Diabetes Study
ICRs	Islet Cell Resource Centers
ITN	Immune Tolerance Network
MMPC	Mouse Metabolic Phenotyping Centers
NHPCSG	Non-Human Primate Transplantation Tolerance Cooperative Study Group
SEARCH	Search for Diabetes in Youth Study
T1DGC	Type 1 Diabetes Genetics Consortium
T1D-RAID	Type 1 Diabetes-Rapid Access to Intervention Development
TEDDY	The Environmental Determinants of Diabetes in the Young
TrialNet	Type 1 Diabetes TrialNet
TRIGR	Trial To Reduce IDDM in the Genetically at Risk

Other Acronyms and Abbreviations

ARB	angiotensin receptor blocker
ACE	angiotensin converting enzyme
AGE	advanced glycation endproducts
ARE	antioxidant response element
AMP	adenosine monophosphate
ATP	adenosine triphosphate
BB	biobreeding
BGAT	blood glucose awareness training
BMI	body mass index
BOLD-fMRI	blood oxygen level-dependent functional magnetic resonance imaging
CGMs	continuous glucose monitors
ChIP	chromatin immunoprecipitation
DMK	dystrophia myotonica kinase
ES cell	embryonic stem cell
ESRD	end-stage renal disease
FACS	fluorescence activated cell sorting
fMRI	functional magnetic resonance imaging
GAD	glutamic acid decarboxylase
HAAF	hypoglycemia-associated autonomic failure
HbA1c	hemoglobin A1c
HLA	human leukocyte antigen
HMGB1	high mobility group box 1
HTS	high-throughput screening
IA-2	anti-tyrosine phosphatase
ICA	islet cell autoantibodies

IDDM	insulin-dependent diabetes mellitus
IGRP	islet-specific glucose-6-phosphatase catalytic subunit related protein
INS	insulin gene
LDL	low-density lipoprotein
Mbp	mega base pairs
MHC	major histocompatibility complex
mRNA	messenger RNA
miRNA	micro RNA
MLI	NIH Roadmap Molecular Libraries Initiative
MODY	Maturity Onset Diabetes of the Young
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
ngn 3	neurogenin 3
NIP	Nutritional Intervention to Prevent Type 1 Diabetes
NKT	natural killer T cells
NOD	non-obese diabetic
PET	positron emission tomography
PPAR-alpha	peroxisome proliferator-activated receptor-alpha
PTPN22	protein tyrosine phosphatase N22 gene
RAGE	receptor for advanced gylcation endproducts
RNAi	RNA interference
ROS	reactive oxygen species
SACO	serial analysis of chromatin occupancy
siRNA	small interfering RNA
SNP	single nucleotide polymorphism
TNF	tumor necrosis factor
VEGF	vascular endothelial growth factor
VNTR	variable number of tandem repeats

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