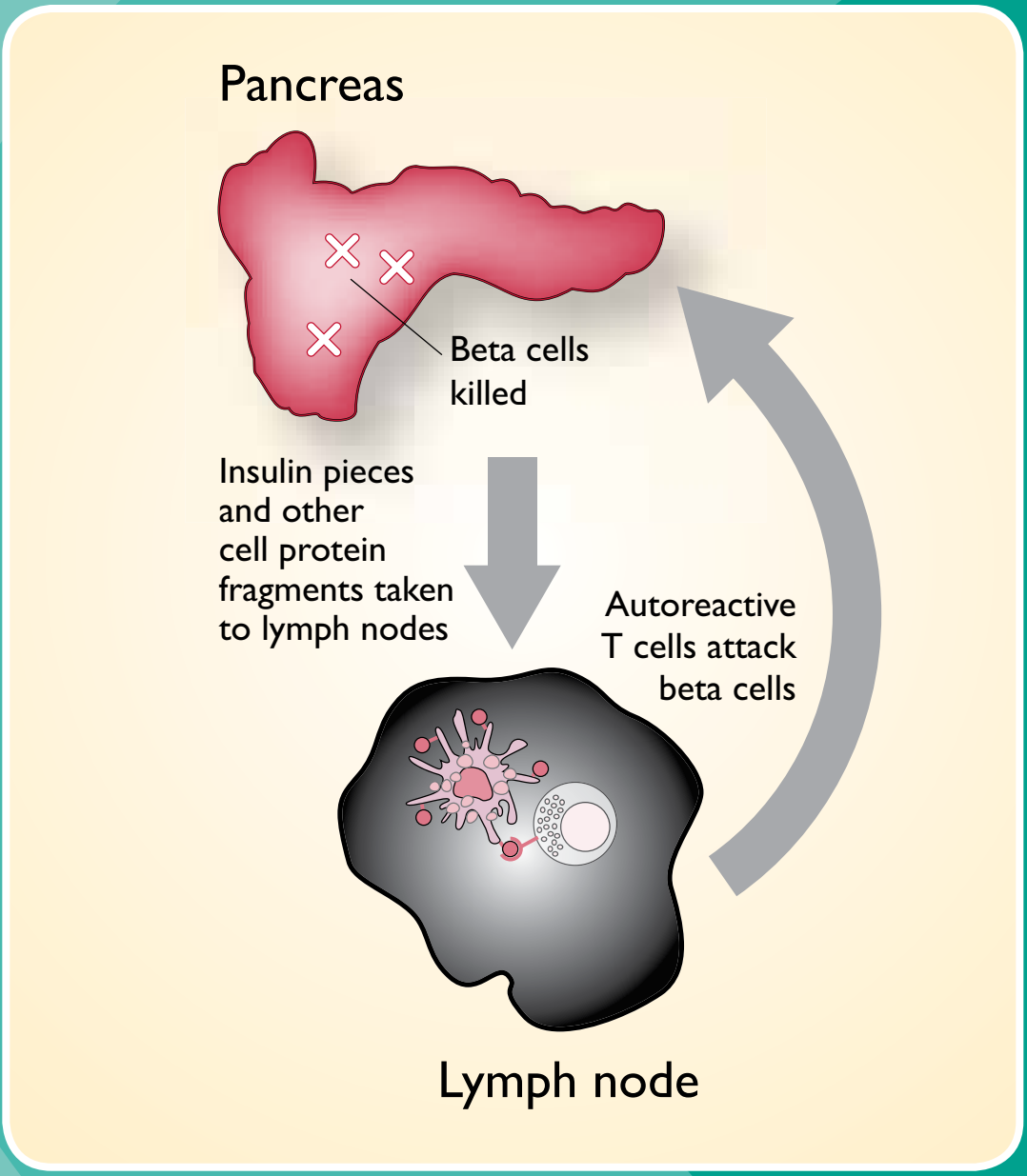


The cycle of autoimmune attack in type 1 diabetes. Incited by various signals, immune T cells launch a misguided attack on beta cells in the pancreatic islets. Destruction of beta cells releases proteins, including insulin, that reinforce the autoimmune response. (*Image courtesy of Focus, Harvard Medical School.*)

TYPE 1 DIABETES AND AUTOIMMUNITY

contents:

INTRODUCTION

Each year, thousands of children and young adults are diagnosed with the autoimmune disease type 1 diabetes—usually without warning. By the time they are diagnosed, their insulin-producing beta cells have been partially or completely destroyed. Without insulin, patients lose the ability to regulate uptake of dietary glucose into cells and tissues—effectively starving the body while blood glucose levels continue to rise. Patients are faced with a lifetime of insulin replacement therapy, administered via shots or an insulin pump, to control blood glucose levels; bouts of dangerously low or high blood glucose; and the threat of long-term health complications. While research supported by the NIH, other Federal agencies, voluntary organizations, and industry has made it possible for people with type 1 diabetes to live longer and healthier lives, the overarching research challenge remains to elucidate the disease, thwart its development and progression, and ultimately arrive at a cure. Fostering discovery in type 1 diabetes diagnosis, development, and the role of the immune system remains key to overcoming this research challenge.

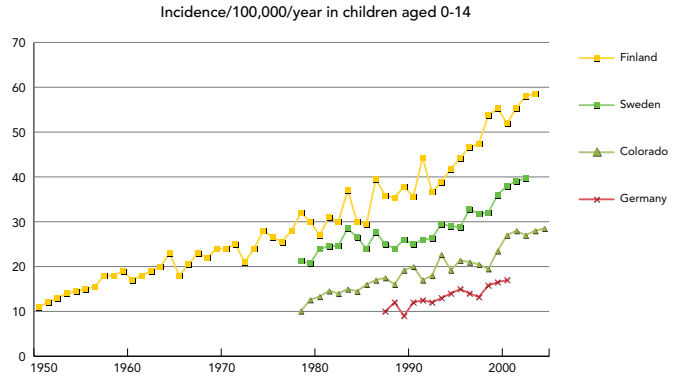
When the symptoms of type 1 diabetes strike, it is a late stage event in a progressive, misguided destruction of the beta cells by the immune system—a process called autoimmunity. Normally, the immune system protects the body against infectious disease and cancer by destroying pathogens and tumor cells. In autoimmunity, a sub-population of the immune system T cells escapes a natural process of elimination by the body and instead can go on to incite tissue damage and disease. In persons who are genetically susceptible to developing type 1 diabetes, these rogue cells migrate to the pancreas, zeroing in on the cell clusters called islets where beta cells reside. There, they can spark an immune system response that eventually destroys the beta cells, resulting in the need for insulin replacement therapy.

The misguided destruction sparked by autoreactive T cells in type 1 diabetes targets insulin and other normal, "self" beta cell proteins as if they belonged to dangerous microbes. For example, antibodies generated by the autoimmune response (autoantibodies) neutralize healthy beta cells bearing target protein(s) (antigen). A key objective of research to prevent or reverse type 1 diabetes involves finding a way to re-instill immune tolerance—the process by which the immune system considers a protein or other molecule as self, and does not mount a destructive response against cells or tissues containing that protein. This could require, for example, removing the rogue T cells, or forcing them into a non-reactive state. Several NIH-funded multi-center clinical trial networks are involved in major research efforts focused on solving this problem, including, but not limited to, the Immune Tolerance Network (ITN), the Type 1 Diabetes TrialNet, and the Cooperative Study Group for Autoimmune Disease Prevention. The induction of tolerance could, in theory, block the autoimmune process underlying type 1 diabetes. Thus, tolerance has been at the basis of design of many promising, new strategies to combat this disease.

Testing blood glucose. Although it can strike at any time, type 1 diabetes most often strikes in youth and young adulthood, leading to a lifetime of blood glucose testing and insulin administration, via shots or a pump, to manage the disease and prevent or delay serious health complications. *(Photo credit: © iStockphoto.com/MarkHatfield)*

Indeed, a series of cell- or drug-based therapies, some of which are already in use for other medical conditions, are being tested both alone and in combination. Because people with type 1 diabetes are prone to developing other autoimmune diseases as well, researchers are also seeking clues to type 1 diabetes in the overlap and distinctions between these diseases.

Ideally, effective therapies to induce tolerance should selectively halt harmful immune processes without requiring lifelong suppression of a patient's entire immune system. For this reason, researchers are working toward so-called "antigen specific therapies," which would disrupt a particular defect in the immune system (e.g., a misguided response to a protein or other molecule), and are also designing studies to determine whether short-term immune suppression will allow resetting of the body's immune regulation and thus amelioration of beta cell destruction over time. Such efforts are the focus, for example, of the Type 1 Diabetes TrialNet multi-center clinical trials network. Although T cells are generally considered to represent the primary mediators of type 1 diabetes, clinical trials are also testing treatments that target the immune system's B cells—e.g., anti-CD20 therapy—as there is increasing evidence that these cells play a role in promoting disease.



Incidence of type 1 diabetes in children, from neonates to age 14 years, is rising 3 to 5 percent per year. NIH-funded research studies, such as TEDDY, are being pursued to identify the environmental factors responsible. *(TEDDY Study Group (2008). The Environmental Determinants of Diabetes in the Young (TEDDY) Study. Ann NY Acad Sci 1150:1-13. © 2008, John Wiley and Sons. Reprinted with permission.)*

While such trials are under way, research on the causes of type 1 diabetes is also moving forward. Scientists are striving vigorously to understand the interactions between the environment and the immune system, as well as the means by which genes influence immune responses resulting in autoimmunity. The ongoing The Environmental Determinants of Diabetes in the Young (TEDDY) study is an important part of this effort. Interest has also grown in terms of identifying the roles that more "primitive" or less specific arms of the immune system, including innate immunity and inflammation, may play as contributors to the complex pathogenesis of this disease (see sidebar, "Inflammation and Immunity: Reaching into All Diabetes"). For example, evidence is growing for the role of inflammatory cells, such as mast cells and neutrophils, and cells of the innate immune system, such as NK or natural killer cells, in type 1 diabetes pathogenesis. Interest has also been sparked in the possible role of a small population of cells of the

immune system that seem to bridge non-specific and specific immunity (the natural killer T cells, or NKT cells). At the same time, scientists are studying the role of other important immune system cells, including dendritic cells, which are specialized antigen-presenting cells that can either activate immunity or induce self-tolerance. The target tissue for the destructive process, the pancreas, has also seen a marked renewal in research interest after lying fallow for a period of decades. This effort has provided much in the way of understanding the natural history of beta cell destruction in the disease and, when combined with the efforts in cell biology on beta cell regeneration (see "The Beta Cell" chapter), provides hope for novel avenues of reversing type 1 diabetes in those with established disease.

The multi-faceted research efforts already under way to help bring about an end to the burden of type 1 diabetes continues to be shaped by new knowledge and discovery. The remainder of this chapter focuses both on major research advances and on new and emerging opportunities for research on type 1 diabetes and autoimmunity that could lead to achieving this important goal.

RECENT RESEARCH ADVANCES

In just the past several years, scientists have learned a great deal about the immune system and how its normally protective functions go awry in type 1 diabetes and other autoimmune diseases. Technological developments have enabled scientists to image cells and tissues in living organisms. These advances have accelerated other, clinical efforts to develop therapeutic approaches to prevent, reverse, or treat type 1 diabetes. The following are some major examples of research that has advanced understanding of type 1 diabetes and autoimmunity.

Islet Cell Transplantation To Reverse Long-Standing Type 1 Diabetes Continues To Show Promise: After years of attempts, reproducible insulin independence has been achieved in humans receiving islet transplants as treatment for type 1 diabetes. Indeed, studies published a decade ago, emanating from Edmonton, Canada, suggested that long-term (more than 2 years) islet survival and maintenance of function could be achieved. A key part of this early success was the adoption of treatment with agents that showed promise in preventing rejection and recurrent autoimmunity without suppressing or altering beta cell function. Even partial graft function allows for maintenance of improved metabolic control in clinical recipients. The so-called "Edmonton protocol" paved the way to many new approaches in islet transplantationcurrently under study to provide for better safety and glycemic control (e.g., new enzymes and procedures for islet isolation, novel drug regimens, and insulin independence following transplant with islets from a single organ donor). For example, a variety of drug regimens are being tested in clinical trials (see Table 1). Researchers are also testing the use of agents that interfere with inflammation and coagulation in the early post-transplant period

to determine if they can augment islet engraftment and survival. Such trials will also inform attempts to interfere with pathogenesis just before or just after diagnosis, as it is expected that agents that interfere with re-emergence of autoimmunity in the transplant could also be used to treat the primary autoimmunity underlying type 1 diabetes.

New Studies of Promising Therapeutics Spurred by Clinical Trials that Assessed Safety and Efficacy of Treatments To Prevent or Delay Type 1 Diabetes: Multiple agents have been tested in people at risk for developing type 1 diabetes to examine their effect on preventing or reducing the incidence of the disease. Completed trials include tests of nicotinamide, as well as injectable, nasal, and orally delivered insulin. While some of these agents failed to show a direct beneficial effect overall, the oral insulin trial within the Diabetes Prevention Trial-Type 1 (DPT-1) conducted an *ad hoc* analysis of a subpopulation of treated participants who had higher levels of autoantibodies against insulin and found a significant (4 year) delay, on average, in diabetes onset in these patients—a finding currently being replicated in a trial conducted by the Type 1 Diabetes TrialNet. Furthermore, in people with newly-diagnosed type 1 diabetes, therapeutic agents have been found that preserve beta cell function, as evaluated by measurement of C-peptide (a by-product of insulin production). These findings are leading the way to new trials to prevent or delay the disease in people at risk for type 1 diabetes (see Tables 2 and 3).

Table 1: Drug Trials in Islet Transplantation. A selection of ongoing or recently completed trials. Please see *www.clinicaltrials.gov* for more information.

Agent	Mechanism/Target (if known)	Clinical Trial Phase for Islet Transplantation Use
Anti-LFA-1, efalizumab (Raptiva®)	Monoclonal anti-CD11a	I-II
Anti-CD3, teplizumab	Monoclonal anti-CD3	I-II
Mutant CTLA-4Ig, belatacept	Co-stimulatory blockade	II
Extendin-4 (Byetta®)	GLP-1 like activity for beta cell protection/growth	I-II
$CD34^+$ cells	Stem cell immune modulation	I-II
TNF alpha antagonist		
1. Infliximab (Remicade®)	1.TNF alpha monoclonal antibody	1. I-II
2. Etanercept (Enbrel®)	2.TNF receptor 2-human IGG1 fusion protein	2. I-II, III
Anti-CD52, alemtuzumab (Campath-1H®)	Monoclonal T-cell depleting antibody	I-II
Anti-thymocyte globulin (Thymoglobulin®)	T cell depletion	I-II, III
Deoxyspergualin, gusperimus	Anti-inflammatory and immunosuppressive through inhibition of NFkappa B signaling and T/B cell antigen presenting cell differentiation	II
Anti-CD20, rituximab (Rituxan®)	B cell depletion	II
Sirolimus (Rapamune®)	mTOR inhibitor	I-II, III
Tacrolimus (Prograf®)	Calcineurin inhibitor	I-II, III
Mycophenolate mofetil (CellCept®)	Inosine monophosphate dehydrogenase inhibitor	I-II, III
Cyclosporine A (Neoral®)	Calcineurin inhibitor	I-II, III
Mycophelolate sodium (Myfortic®)	Inosine monophosphate dehydrogenase inhibitor	I-II, III
Daclizumab (Zenapax®)	Monoclonal anti-IL-2 receptor antibody	I-II, III
Basiliximab (Simulect®)	Monoclonal anti-IL-2 receptor antibody	I-II, III
Abatacept (Orencia®)	Co-stimulatory blockade	I-II
Lisofylline	Anti-inflammatory	II
Everolimus (Certican®)	mTOR inhibitor	I-II
Low dose corticosteroids	Anti-inflammatory	I-II, III
Low molecular weight dextran sulfate	Innate immune response inhibitor	II
IL-1-antagonist IL-1 Ra, anakinra (Kineret®)	IL-1 receptor antagonist	I-II
Anti-CD2, alefacept (Amevive®)	Anti-CD2 (memory T cells and NK cells)	I

Table 2: Agents Under Study for Reversal of Type 1 Diabetes. Listed in the table is a selection of ongoing or recently completed trials. Please see *www.clinicaltrials.gov* for more information.

Agent	Mechanism/Target (if known)	Clinical Trial Phase
Anti-CD3, teplizumab and otelixizumab (Tolerx®)	T cell immunomodulation	II-III
GAD alum (Diamyd®)	Antigen-specific tolerance vaccination	II-III
HSP60 (DiaPep277®)	Antigen-specific tolerance vaccination	II-III
Anti-CD20, rituximab (Rituxan®)	B cell depletion	II
CTLA4Ig, belatacept	Costimulation blockade	II
Anti-thymocyte globulin (Thymoglobulin®)	T cell depletion	II
Interleukin-1 (IL-1) pathway antagonist, anakinra (Kineret®)	Anti-inflammatory and improve beta cell survival	II
Alpha-1-antitrypsin (Aralast®)	Anti-inflammatory	II
Adult human mesenchymal stem cells (Prochymal™)	Impart immune regulation	II
GAD alum (Diamyd®), lansoprazole (Prevacid®), sitagliptin (Januvia®)	Enhancing GLP-1 and gastrin function for beta cell protection/growth, antigen specific immunomodulation	II
Closed-loop metabolic control at diagnosis	Intensive glucose control spares beta cell function	II
Imatinib (Gleevec®)	Protein tyrosine kinase inhibitor	II (pending)
Insulin B-chain	Antigen-specific tolerance vaccination	I-II (pending)
Statin, atorvastatin (Lipitor®)	Anti-inflammatory and lipid modulation	I
T regulatory cell therapy	Augment regulatory T cell numbers	I (pending)
Autologous dendritic cell therapy (with and without peptides from beta cell antigens)	Tolerogenic vaccine	I
Interleukin-2 (IL-2) (Proleukin®) and sirolimus (Rapamune®)	Downregulates T effector while sparing T regulatory function	I
Umbilical cord blood infusion	Enhance T regulatory cell numbers	I
Granulocyte-colony stimulating factor (Neulasta®)	Enhance T regulatory cell numbers	I
Anti-thymocyte globulin (Thymoglobulin®), granulocyte-colony stimulating factor (Neulasta®)	T cell depletion, enhance T regulatory cell numbers	I-II
"PowerMix" (Mixture of two Ig-cytokine fusion molecules, one an IL-2 agonist the other an IL-15 antagonist)	Suppression of T effectors while sparing T regulatory function	Pre-clinical/ I
Interleukin-1 (IL-1) receptor, canakinumab (Ilaris®)	Anti-inflammatory	II (pending)
Bayhill's DNA vaccine 3021 (proinsulin)	Antigen-specific tolerance or regulation induction	I-II
Anti-CD2, Alefacept (Amevive®)	Anti-CD2 (memory T cells and NK cells)	I-II (pending)
Inhibitor of interleukin-1beta, XOMA 052	Anti-inflammatory	II

New Markers Discovered for Identifying Type 1 Diabetes-Susceptible Individuals Prior to Disease Onset: Scientists recently discovered a new autoantibody that is an excellent additional marker for identifying pre-clinical type 1 diabetes, and improves the ability to predict disease when combined with previously known autoantibodies. With the discovery of this fourth major autoantibody (Zinc Transporter 8 (ZnT8) autoantibody), and analysis of large cohorts of children, autoantibody prediction of type 1 diabetes risk continues to gather strength, with increasing evidence for its feasibility both for relatives of people with type 1 diabetes and, more importantly, for the general population. Approximately 1 million Americans express multiple autoantibodies targeting islet proteins (i.e., rate of 1/300) and are at high risk of progression to type 1 diabetes. Prediction using autoantibodies, combined with increasing refinement of genetic and metabolic prediction, sets the stage for prevention trials at multiple stages of the disease.

Identification of Key Self-Antigens in Type 1 Diabetes: Scientists have learned that insulin is a critical antigen for the development of type 1 diabetes in non-obese diabetic (NOD) mice, and have identified several other antigens recognized by autoreactive $CD4^+$ T cells, $CD8^+$ T cells, or by autoantibodies in mice or humans (several of them shared between the two

Table 3: Agents Under Study for Prevention of Type 1 Diabetes. Listed in the table is a selection of ongoing or recently completed trials. Please see *www.clinicaltrials.gov* for more information.

Agent	Mechanism/Target (if known)	Clinical Trial Phase
Oral insulin	Oral antigen specific tolerance	II
Omega-3 fatty acids	Anti-inflammatory	Pilot
Hydrolyzed milk	Unknown	II
Nasal insulin	Mucosal antigen specific tolerance	II
Oral and nasal insulin	Oral and mucosal antigen specific tolerance (prePOINT study)	Pilot
Anti-CD3, teplizumab	Monoclonal anti-CD3	II

species). Other proteins resident to beta cells, including islet-specific G6Pase-related protein (IGRP), glutamic acid decarboxylase (GAD), and ZnT8, continue to garner much research interest for the potential roles they may have in disease development, as well as in the design of antigen-specific therapies to prevent and/or cure the disease. Indeed, one of these molecules (GAD) is undergoing phase III testing in both the United States and Europe for its ability to prevent or reverse type 1 diabetes when administered as a vaccine. In addition, recent studies suggest the immune system's responses to these antigens—in particular, autoantibody profiles in humans—correlate well with disease parameters, encouraging their expanded use in clinical trials as a means to monitor and define success.

Continued Improvement in Understanding Natural History of Type 1 Diabetes in Humans: Multiple studies in children and adults have provided insights into how type 1 diabetes develops. Hundreds of thousands of children have now participated in prospective studies across the world identifying genetic and autoantibody risk for type 1 diabetes, with highest risk children then followed to development of diabetes. Children participating in these clinical studies have benefited by being diagnosed earlier and having lower rates of a life-threatening condition called diabetic ketoacidosis at type 1 diabetes onset. Increasing numbers of younger children are being enrolled into clinical trials and studies, mostly from families with prior incidence of the disease (see the "Special Needs for Special Populations" chapter); the larger number of young children in trials creates a special challenge, as efforts seeking to prevent type 1 diabetes in young children require the highest ethical standards for safety. On the cellular level, many T cell and cytokine abnormalities evident prior to diabetes and correlated with pathogenesis have been defined. While research continues in that area, it is also now clear that a subset of patients with type 1 diabetes preserves some beta cells for decades—some have distinct and poorly characterized forms of type 1 diabetes, and others have immune-mediated type 1 diabetes that is either progressing slowly or in whom the process of beta cell destruction has naturally halted. Finally, findings from genetic studies highlight the fact that many susceptibility genes for type 1 diabetes are shared with other autoimmune diseases (e.g., thyroid disease, multiple sclerosis, celiac disease), which has important implications for pathogenesis and therapy (see also the chapter on "Genetic Basis of Type 1 Diabetes, Type 2 Diabetes, Obesity, and Their Complications").

Recognition of the Importance of Innate and Adaptive Immunity in both Type 1 and Type 2 Diabetes: Scientists have gained a greater understanding of certain immune cells, called alternatively activated macrophages, that may play a role in type 1 diabetes, as well as in the obesity-induced inflammation and insulin resistance associated with

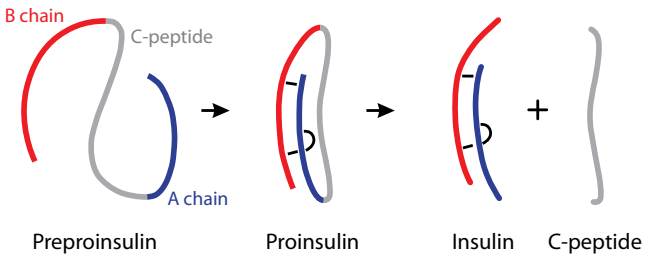
type 2 diabetes. The chemical signals (cytokines) that some of these cells produce may be protective against disease. In addition to the protective role of macrophages and other cells of the innate immune system, there is a growing interest in understanding the role of the adaptive immune system in pathogenesis of type 2 diabetes. The adaptive immune system involves the T cell and autoantibody responses to "self" molecules. Researchers have now observed that as many as 10 percent of individuals with type 2 diabetes have evidence of adaptive immune system activity. These are just two examples of a growing appreciation of the importance of immune responsiveness in type 2 diabetes, and how it may form the basis for future areas of therapeutic intervention. The potential importance for innate immunity in type 1 diabetes also derives from recent studies of the gut microbiome, the microbial communities that line the intestine. Studies in animal models have shown the pronounced ability for gut bacteria to influence innate immune responses that may have an impact on type 1 diabetes.

Animal Models Advance Understanding of Type 1 Diabetes and Testing of New Therapies: The spontaneously diabetic NOD mouse has provided abundant insights into the pathogenesis of type 1 diabetes. Studies of NOD mice have led to the identification of many of the targets of the autoimmune response, as well as the cell populations that mediate destruction of the insulin-producing beta cells. These insights have led to identification of similar targets and destructive cell populations in humans with type 1 diabetes. The NOD mouse model has also been valuable in understanding how diabetes-susceptibility genes and genetic networks common in mice and humans affect the immune response that leads to disease. Moreover, for a limited but growing number of therapeutics, reversal of diabetes in new-onset, spontaneously diabetic NOD mice is possible. As a result, early diabetes reversal in NOD is increasingly used as a model for testing the potential efficacy of therapeutics in clinical trials. In the NOD mouse, reversal has been found to depend on intervention soon after diabetes onset, when there is still enough residual functional beta cell mass able to recover if the autoimmune process is halted. While not all therapies that work in animal models show such an effect in humans, others (e.g., anti-CD3 and oral insulin) do appear promising. Hence, animal models have clearly proven themselves useful for finding strategies to reverse disease in people with type 1 diabetes.

Development of Sophisticated Mouse Models of Human Disease for Study of Type 1 Diabetes: Increasingly sophisticated stocks of mice modeling human disease have been developed that are now providing the means to understand clinically relevant components of type 1 diabetes pathogenesis. These include mice that are either engrafted with functional human cells or tissues, genetically engineered to express human genes, or both, and can recapitulate aspects of the pathogenic process. These mice have been used to identify targets of the human immune response against transplanted islets, and have led to insights into the destructive cell populations that are important to this process. Mice engrafted with functional human immune systems may permit certain human immune responses, including autoimmune responses, to be manipulated in small animal models. As these types of studies cannot be done in people, such mouse models could facilitate the conduct of important translational research, providing insights into safety and efficacy before enrolling participants in clinical trials.

Beta Cells Still Present at Onset of Type 1 Diabetes: In contrast to earlier assumptions, researchers have found that, at diagnosis, a majority of

people with type 1 diabetes have circulating C-peptide, a marker of insulin production by the pancreas. While C-peptide levels are reduced in comparison to people without the disease, the measurable and inducible C-peptide is very suggestive of functional beta cell mass. This observation is important for future therapies as the positive benefit of immune modulation in the NOD mouse is best realized when a pancreatic beta cell mass capable of promoting euglycemia is present. Moreover, in humans the presence of C-peptide has been associated with improved control of diabetes and less risk of life-threatening hypoglycemia. Thus, preservation of C-peptide, as well as expansion of beta cell mass in new-onset type 1 diabetes, is a major focus of therapeutic investigation.



The hormone insulin is formed by chemical modification and cleavage of a precursor molecule. The cleaved "C-peptide" is useful for monitoring residual beta cell function in people with diabetes who are on insulin therapy.

Beta Cells Can Resist Immune Mediated Destruction: Scientists have defined some of the pathways by which beta cells are destroyed by the immune system. In addition, gene therapy and transgenic experiments in animal models have shown that beta cells can be protected from cytokine induced destruction as well as transplant rejection. This research has shed light on potential therapeutic strategies to preserve beta cell function that can be tested in people in the future.

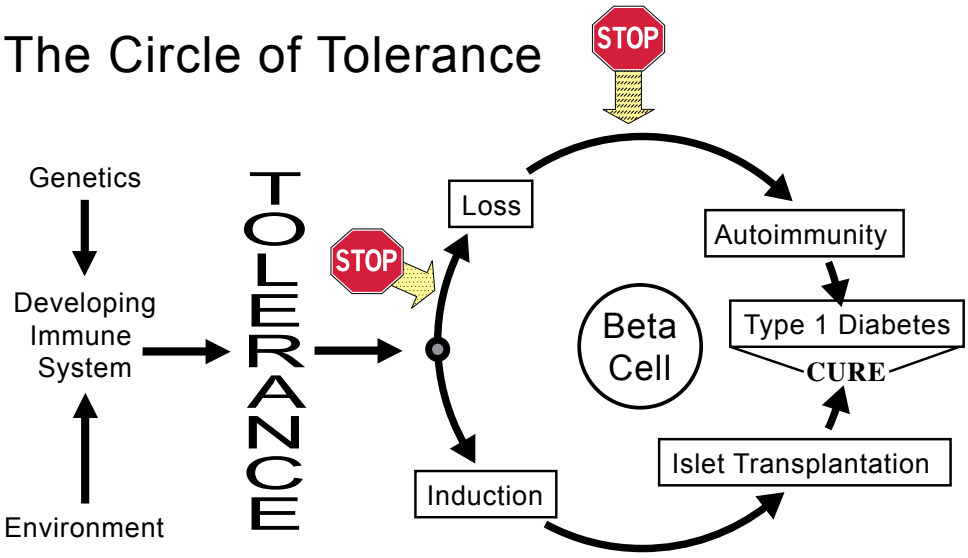
Elucidating Mechanisms Underlying Tolerance: Type 1 diabetes is thought to arise from a defect in immune tolerance, the "normal" state in which the immune system is non-reactive to healthy cells and tissues. Scientists have learned much about the cellular and molecular mechanisms controlling tolerance induction in recent years. In particular, some gene expression regulators (transcription factors, e.g., forkhead box p3 (Foxp3)) are important for the proper function of regulatory T cells, which suppress misdirected immune responses, while other transcription factors (e.g., autoimmune regulator (Aire)) function to allow the removal of autoreactive T cells during development. Other factors known to be important for tolerance induction or maintenance include those involved in immune cell signaling or modulating immune responses (co-stimulatory molecules and cytokines); the biology of regulatory T cells; and the function of dendritic cells, a type of antigen presenting cell, in the process. This knowledge has enabled the design of several successful strategies for imposing a state of tolerance, for example, to transplantation antigens in normal rodents, but this has proven to be much more challenging for restoring self-tolerance in rodent models of diabetes. Numerous observations suggest that similar deficiencies in tolerance induction play an important role in human type 1 diabetes, including the association between alleles of the gene encoding insulin (*INS*), their expression level in the thymic stroma (where removal of autoreactive T cells takes place), and diabetes incidence; the development of diabetes in patients with mutations in the genes encoding Aire (*AIRE*) and Foxp3 (*FOXP3*); and the observations of defective regulation of activated T cells by regulatory T cells in type 1 diabetes patients. These findings are helping pave the way to

future approaches that could restore a state of immune tolerance in people with type 1 diabetes.

Understanding Mechanisms Contributing to the Initiation of Insulitis in Type 1 Diabetes: Scientists have made progress in elucidating the molecular mechanisms underlying insulitis in animal models of type 1 diabetes. Insulitis, the inflammation of the pancreatic islets that leads to beta cell destruction, is marked by the appearance of white blood cells (T cells, B lymphocytes, and others) in the islets. Studies in several rodent models have now established that potentially diabetogenic T cells circulate innocuously through the blood and lymphoid organs until a poorly understood event early in life provokes islet beta cell death, releasing antigens that stimulate the islet-antigen-reactive T cells and cause them to be retained in the pancreas, where they create structures resembling lymphoid tissue. It is not yet known to what extent this scenario is followed in humans, although insulitis similar to that of rodent models has been observed in sections of pancreata from at least some people with type 1 diabetes. These findings in rodent models could help researchers identify the initial steps that activate the T cells responsible for launching autoimmune destruction of beta cells in humans and lead to new targets for intervention.

Discovery of Multiple Cellular Factors That Contribute to Progression to Diabetes: The major culprits in type 1 diabetes pathogenesis in rodents are "helper" T cells ($CD4^+$ T cells) that promote immune system activity and/or cytotoxic T cells ($CD8^+$ T cells) that actually destroy beta cells. There is evidence that this is also true for human type 1 diabetes, because patients have responded to treatment with therapeutic agents directed against T cell activity, such as cyclosporine-A and anti-CD3 monoclonal antibody. Nonetheless, there has been increasing appreciation of the importance of other cellular players, notably macrophages, dendritic cells, B cells, natural killer (NK) cells, and NKT cells. Recent years have also brought an increasing understanding at the molecular level, particularly regarding the role of co-stimulatory molecules that transmit either stimulatory or inhibitory signals to T cells (e.g., CD28, CTLA-4, PD-1) and the role of cytokines (e.g., type-1 and -2 interferons, IL-2, IL-21, IL-15, IL-10, IL-17). However, more remains to be learned, as scientists have not yet been able to integrate the various cellular and molecular elements into a coherent disease scenario. In particular, although a number of candidate cells and molecules have been identified, there is no consensus on what agents ultimately destroy the beta cells, perhaps because different mechanisms come into play in different models and in different patients. The discovery of multiple factors that could play a role in type 1 diabetes is important for people with the disease, as these factors may represent additional targets for therapeutic intervention.

Immunoregulation—Understanding Cellular Controllers: It has become clear that type 1 diabetes is a regulated disease: initiation of insulitis and the time between insulitis and conversion to overt diabetes



The circle of tolerance. Tolerance denotes the absence of a detectable, functional immune response in the absence of immunosuppression. Loss of tolerance is at the root of autoimmunity. In type 1 diabetes, preventing this loss of tolerance (dotted "stop") is the ultimate goal, to prevent disease. Current efforts are also aimed at interfering with T cell function after the loss of tolerance (striped "stop"), e.g., with immunosuppressive agents or other means. Strategies to induce tolerance are also needed to aid success of islet transplantation and potentially provide a cure. *(Image courtesy of Dr. Aldo A. Rossini. Adapted from Diabetes, Vol. 53, 2004; 267-275. Copyright 2004 American Diabetes Association. Reprinted with permission from the American Diabetes Association.)*

are both subject to immunological control. In recent years, immunologists have identified and characterized a number of cellular controllers. The most well-studied are regulatory T cells (also called "Tregs") expressing the Foxp3 protein marker, but appreciation is growing for the activities of regulatory B cells, monocytes, and other T cell populations. The importance of Foxp3-expressing regulatory T cells in regulating diabetes progression has been established in a number of rodent models. Precisely when, where, and how these cells act is still actively debated. The fact that people who have a rare disease called "immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome" (IPEX), caused by a mutant *FOXP3* gene, rapidly develop severe type 1 diabetes, argues for the importance of regulatory T cells in controlling human diabetes. It remains controversial whether or not people with classical type 1 diabetes have a defect in regulatory T cell-mediated immunoregulation. In either case, modulating regulatory T cell activity represents an interesting therapeutic strategy that is being actively pursued.

INFLAMMATION AND IMMUNITY: REACHING INTO ALL DIABETES

Imagine receiving a diagnosis of type 2 diabetes. The symptoms seem to add up—fatigue, dizziness, thirst—and medical tests reveal high blood glucose, but apparently no need for insulin. Middle-aged and a bit overweight, the diagnosis seems clear, and you start a standard program of control with diet and exercise plus oral agents. Although your diabetes is initially well-controlled, within a few years your glucose levels have become high again. Frustrated, you seek new tests—and receive a new diagnosis, one of autoimmune diabetes that now must be treated with insulin. As it turns out, distinguishing different forms of diabetes can be less straightforward than commonly thought, and autoimmune diabetes can strike at any age. At the same time, research is revealing that type 1 and type 2 diabetes may have more in common than previously thought, an observation that is opening up new avenues to therapy.

Traditionally, doctors have distinguished between type 1 and type 2 diabetes according to certain clinical signs or symptoms. The autoimmune disease type 1 diabetes is more likely to be suspected in children and youth, and diagnosis is confirmed if a person has autoantibodies and is insulin-dependent at diagnosis. Conversely, people who are middle-aged and older and/or obese are more likely to be diagnosed with the non-autoimmune disease type 2 diabetes, which is characterized by insulin resistance that at first can generally be treated with diet, exercise, and oral medication. In recent years, however, some of the traditional lines between type 1 and type 2 diabetes have blurred. More children have been developing type 2 diabetes, associated with the epidemic of obesity. In addition, given the increases in obesity in the population, many children with type 1 diabetes are overweight at diagnosis—making it difficult to distinguish between type 1 and type 2 diabetes in youth based on weight alone.

Furthermore, in between these two classic types of diabetes lies a grey area, populated largely by people diagnosed as adults. Some people may experience an unusual, late onset of type 1 diabetes, requiring immediate treatment with insulin. Others show signs of both type 1 and type 2 diabetes, a condition called latent autoimmune diabetes in adults, or LADA. While people with this form of diabetes are often diagnosed with type 2 diabetes because they don't need insulin initially, they are actually suffering from an autoimmune process that damages and destroys the insulin-producing beta cells. Fortunately, once suspected, this confusion can be resolved with testing for islet autoantibodies. As many as 10 percent of newly-diagnosed, non-insulin-requiring adults with diabetes may have this form of diabetes. Similarly, autoantibodies are sometimes found in children and youth thought to have type 2 diabetes (latent autoimmune diabetes of youth—LADY), but less is known about how many people are affected in this age group.

Clinicians, patients, and researchers alike struggle with this apparent overlap of types of diabetes. For patients and clinicians, obtaining a correct diagnosis

can be a challenge, and there are still therapeutic hurdles once a diagnosis is achieved—for example, there is still debate about the optimal management strategy for LADA. For researchers, an important goal is to better understand why some people develop classic type 1 diabetes in youth, others when they are adults, and what are the underlying causes of differences in disease course in older and younger people with classic type 1 diabetes and in people with LADA and other hybrid forms of diabetes. There may, in fact, be a spectrum of autoimmune diabetes, of which classic type 1 diabetes is the most extreme form. Genes, age, differences in production of autoantibodies, and environmental factors may all play a role. People with diabetes would benefit from an improved classification of type 1 diabetes that would highlight differences and potentially lead to more tailored approaches to therapy.

Encouragingly, new studies of how diabetes develops and progresses are uncovering shared features between type 1 and type 2 diabetes that may also help explain some intermediate forms of the disease. At the heart of these studies are inflammation and immunity. Inflammation is a complex mixture of cellular and chemical responses the body deploys in reaction to perceived cell and tissue injury, such as a bacterial infection. While useful to help control infections and promote tissue repair, inflammation is a blunt instrument that can also inflict harm on healthy tissues, especially if it is misdirected or becomes chronic. Different types of inflammatory reactions have been linked to different types of diabetes, but now there is evidence that these lines may not be so distinct. For example, inflammation of the islets (insulitis)—the hallmark of type 1 diabetes—is not exclusive to type 1 diabetes and may contribute to loss of beta cells in type 2 diabetes. Conversely, low-grade systemic inflammation and insulin resistance contribute to type 2 diabetes, but may also play a role in type 1 diabetes, exacerbating the dominant autoimmune process that is destroying beta cells. Inflammation is also implicated in the development of diabetes complications.

Cells of the immune system both promote inflammation and are vulnerable to its effects. Scientists suspect that some of the clues to variations in diabetes lie in a complex interplay among cells involved in inflammation, those implicated in autoimmunity, and inflammatory signals themselves. These interactions, in turn, will be affected by genetic and environmental factors. Learning more about how inflammation is regulated by the immune system in the context of diabetes could reveal more about the similarities and differences between type 1 and type 2 diabetes and their complications, potentially reveal more about mechanisms underlying hybrid forms of diabetes, and lead to more specific therapeutic approaches that could benefit people with different types of diabetes.

KEY QUESTIONS AND FUTURE DIRECTIONS FOR RESEARCH

The 1999 report of the congressionally-established Diabetes Research Working Group (DRWG), *Conquering Diabetes: A Strategic Plan for the 21st Century*, recognized the importance of understanding the immune system in winning the battle against type 1 diabetes. Since the publication of that roadmap for diabetes research, recommended steps—including exploiting important discoveries from the fields of immunology and cell and molecular biology in order to find ways to prevent or block development of diabetes, and establishing multi-center clinical trials networks to test new therapies—have led to new insights into ways to prevent, treat, or potentially reverse type 1 diabetes. To help speed progress, these efforts have been bolstered and updated through strategic planning processes for the *Special Statutory Funding Program for Type 1 Diabetes Research* and through the 2006 "*Advances and Emerging Opportunities in Type 1 Diabetes Research: A Strategic Plan*." However, the incidence of type 1 diabetes has continued to rise during the past decade, and as prospects for extended life with diabetes improve so does the threat of long-term complications—lending urgency to closing the gaps in the fundamental understanding of type 1 diabetes and autoimmunity while still leveraging what has already been learned. Moreover, the pronounced increases in obesity rates across the age spectrum have led type 1 and type 2 diabetes to become less compartmentalized as diseases in the population (see the "Obesity" chapter), creating an additional challenge to understanding and treating type 1 diabetes. Described below are research questions and opportunities to pursue in the next decade to reach the goal of meeting these challenges.

Human Type 1 Diabetes Trials (Prevention/ Reversal/Transplantation)

Based on continuing research advances over the last decade, major expansions have occurred in efforts designed to prevent or reverse type 1 diabetes, which may in turn lead to effective new therapeutic strategies. Ongoing prevention trials include a pilot study to test orally administered insulin in genetically at-risk people and studies testing nutritional interventions (e.g., vitamin D, fish oil, highly hydrolyzed milk formula, delay in introduction of gluten). Additional clinical trials are planned in this arena, especially to capitalize on agents found to be effective in slowing beta cell loss in new-onset type 1 diabetes by studying their use earlier in the course of type 1 diabetes development and before the diagnosis of disease. Such studies capitalize on new biomarkers of disease and improved predictive models. Clinical trials networks and research consortia have been organized to facilitate collaborations between basic and clinical investigators, promote bench-to-bedside translational research, and test new approaches for preventing the onset of type 1 diabetes, reversing disease, and improving islet transplantation as a treatment option (see also "The Beta Cell" chapter regarding ways to advance islet transplantation). Researchers will benefit from the opportunities presented by these collaborative efforts in developing new studies to answer key clinical questions in type 1 diabetes.

Key Questions

- **Will additional information about genetic underpinnings of type 1 diabetes allow therapies to be targeted to homogeneous populations, thus increasing their effectiveness?**
- **Will antigen-specific versus non-specific tolerance induction protocols be safe and effective in preventing progression to overt type 1 diabetes in individuals deemed to be at high future disease risk?**
- **How can combination therapies using short-course immunosuppressants, cellular mobilization agents, insulin sensitizers, anti-inflammatories, islet antigens, and/or molecules capable of inducing beta cell replication *in vivo* be tested?**
- **How can multi-center, international collaborative trials that support biomarker and discovery studies best be accomplished?**
- **How can very long-term follow-up (i.e., beyond the 1 to 2 year standard for current studies), including metabolic and mechanistic studies, as well as monitoring of adverse events of patients in trials for the prevention of beta cell loss, be accomplished?**
- **Can biomarkers be developed to stratify patients for trials and to obtain an early indication of therapeutic effectiveness?**
- **Will drugs designed for the treatment of other disorders, especially autoimmune disorders, and possessing a highly favorable safety profile, prove efficacious as treatment(s) for type 1 diabetes?**
- **Is it possible that intervention may provide a clinical benefit in patients months or even years after diagnosis?**
- **Could the principles of "disease staging," often used in oncology, be applied to settings of type 1 diabetes both prior to and well beyond the diagnosis of this disease?**

Future Directions

➤ **Conduct coordinated clinical trials to test therapies to prevent or reverse type 1 diabetes.**

Because type 1 diabetes is not a highly prevalent disease, screening of large populations is required to identify an adequate number of persons at risk for the disease and amenable to clinical intervention trials. Clinical trials in people newly-diagnosed with the disease also require a multi-center effort because at present, most (but not all) studies require that people must be recruited within 3 months of onset. Thus, clinical trials in type 1 diabetes require a large cooperative network, which is complex to operate. Coordinated efforts by regulatory agencies (FDA), clinical trial sponsors (NIH and pharmaceutical companies), and participating researchers (institutions) are needed to accomplish trials using combination therapies, which will require many more people than single-agent trials. To facilitate this coordination, more centralization of Institutional Review Boards (IRBs) is needed to allow associated biomarker studies and easier implementation of multi-center trials, and barriers to conducting collaborative, international trials should be addressed.

Bold initiatives are needed to address many of the key questions. For example, early results from disease etiology studies could suggest it would be feasible to perform prevention trials, using benign interventions (e.g., probiotics or other "generally regarded as safe" (GRAS) agents), in very young children, and using surrogate end points and biomarkers of pathogenesis. Such an undertaking would likely require large-scale screening of the general population, combined with registries, in order to help recruiting for prevention trials and to ensure that the results obtained are applicable to the broadest population.

In addition, it is important to note that the design of future clinical trials will need to take into account any beneficial therapies that arise in the interim, which could limit use of placebo controls and complicate trial operations, as well as increase the overall adverse event burden. With this, clinical trials which add therapeutic arms ("rolling") may be needed to efficiently cope with the fast pace of new immunomodulatory drug discovery and rapid drug approvals. This approach could increase the difficulty in recruiting participants for testing newer agents.

Natural History and Pathogenesis of Human Type 1 Diabetes

Researchers have made progress over the past decade in understanding genetic risk factors and development of type 1 diabetes, but much remains to be learned about how—and why—anti-islet autoimmunity takes hold and flourishes in some individuals and not others, especially not in those individuals who carry a protective *HLA* allele, such as *HLA DQB1*0602*. The mechanism by which this allele confers dominant protection is under investigation. The role of environmental factors in the genesis of type 1 diabetes is an area of increasing focus, as understanding these factors and their interaction with individual susceptibilities could lead to new preventive strategies (see the chapter on "Genetic Basis of Type 1 Diabetes, Type 2 Diabetes, Obesity, and Their Complications"). Recent technological developments and large-scale collaborative research efforts already established by NIH will afford scientists the opportunity to meet many of these research challenges in the years ahead.

Key Questions

- **What is the natural history of type 1 diabetes, including the precise sequence of events leading to the initiation of insulitis, and continuing on to clinical diabetes?**
- **Why is type 1 diabetes increasing in incidence and occurring more often at younger ages?**
- **What is the basis of the observed heterogeneity in type 1 diabetes and is there additional heterogeneity yet to be discovered?**
- **Is noninvasive imaging of beta cell mass and associated insulitis achievable?**
- **Can autoimmune pathogenesis at the islet, whether in people in pre-clinical stages of pathology or in autoimmune recurrence in transplant recipients, be measured indirectly in the blood, for example by a measurement of T cell responses to diabetes-relevant antigens? Can such biomarker assays be developed to enhance prediction of type 1 diabetes, facilitate studies of natural history, and serve as surrogate markers in therapeutic trials?**
- **What is the role of the gut microbiome in disease etiology?**

Future Directions

- **Discover triggering factors for islet autoimmunity and environmental factors responsible for the recent increase in incidence of type 1 diabetes.**

Scientists have very little knowledge of the events that initiate anti-islet autoimmunity in humans; knowledge is also limited in animal models that spontaneously develop the disease. In particular, several questions regarding environmental triggers remain unanswered: Is there a specific environmental factor that triggers islet autoimmunity in humans or are there thousands of factors? Is the factor dietary or infectious? Is a triggering factor needed at all? What is the role for the microbiome in the pathogenesis of this disease? These questions represent fundamental gaps in knowledge, but are crucially important to answer because defining triggering factors may permit scientists to design safe strategies for type 1 diabetes prevention. It is likely that the safest prevention of type 1 diabetes on a population basis will come from identifying key environmental factors. Ongoing large NIH studies, such as TEDDY, are screening approximately 400,000 newborns and following those who are genetically susceptible. TEDDY and other studies will hopefully provide the resources for the discovery of key triggering and modulating environmental factors. Depending on the timing and mechanism of triggering, however, even these studies with follow-up of high-risk children at 3 month intervals may not define all the triggering factors. Development of innovative technology and study designs to enhance the ability to define triggers of autoimmunity that may occur over a course of days should be encouraged both as proof of principle in animal models and studied in humans.

- **Better define the heterogeneity and diagnosis of type 1 diabetes and foster the development of therapies specific to different forms of the disease.**

Approximately 15 percent of children at onset of type 1 diabetes lack all islet autoantibodies and, when taken together with studies examining the natural history of type 1 diabetes (i.e., investigations of individuals before hyperglycemic onset), a new appreciation has emerged for the heterogeneity of what is commonly referred to as type 1 diabetes. Some of these children have autoimmune type 1 diabetes, but antibody tests are currently inadequate; some have monogenic forms of diabetes that require tailored therapy; some have forms of type 2 diabetes that are difficult to diagnose; and some are likely to have yet to be discovered genetic forms of diabetes. In addition, heterogeneity may be uncovered only after diabetes diagnosis (see sidebar, "Inflammation and Immunity: Reaching into All Diabetes"). For example, some children may lose residual beta cell function and insulin production more rapidly than other children. Systematic studies of diabetes heterogeneity in children are needed to allow the development of specifically targeted care and to delineate natural history without mixing fundamentally separate disorders. A better understanding of the heterogeneity in the disease at or before diagnosis, as well as after diagnosis, is vital. Indeed, research should address the importance of differences among people in the rate of loss of C-peptide production, and their influences on the rate of developing complications, which could also be influenced by the autoimmune state.

- **Delineate the natural history, or histories, of type 1 diabetes.**

At multiple stages in the development of type 1 diabetes, current knowledge is inadequate for the optimal design of preventive therapy. As more is learned about the genetic determinants of type 1 diabetes, this developing

knowledge needs to be evaluated in terms of how well it may predict development of islet autoimmunity and natural history of progression. Triggering factors are unknown; their identification will almost certainly depend upon being able to define when islet autoimmunity is initiated in a genetically susceptible individual or animal model. Although high rates of progression to diabetes have been demonstrated among multiple-autoantibody-positive individuals, the natural history of long-term progression (more than a decade of follow-up) is unknown. If a subset of individuals escapes progression to diabetes, the existence of "natural escape" will influence therapeutic choices, and the determinants of this state will be important to define. Methods to accurately define beta cell mass in people are needed to improve disease prediction and to define the fundamentals of beta cell loss. Better assays are also needed to define T cell mediated autoimmunity during the period prior to development of type 1 diabetes, when patients are experiencing non-specific symptoms (prodrome), as well as all other phases of pathogenesis. Improved metabolic and physiologic understanding is needed during both the prodrome and post-onset of diabetes, as well as during further loss of beta cells.

- **Elucidate the impact of environmental or other non-genetic factors on development of type 1 diabetes.**

Not all individuals with genetic susceptibility get insulitis, and not all individuals with autoantibodies get diabetes. In fact, the identical twin of a person with type 1 diabetes can take as long as 40 years to develop the disease. Both the age at which islet autoimmunity appears and the rate of progression from autoimmunity to overt diabetes differ for identical twins, with a greater discordance in rate for siblings, and even greater discordance among unrelated individuals in the general population. Type 1 diabetes occurs at all ages, suggesting that precipitating events can occur throughout life. There may also be environmental factors that inhibit rather than promote progression to type 1 diabetes in those at risk for the disease. Innovative application of available technologies, including "next generation sequencing" (e.g., unbiased pathogen detection, intestinal flora, T and B cell receptors, and mutational analysis), messenger microarray, epigenetic analysis, proteomics, metabolomics, and systems biology integration, should be applied to better understand the environmental triggers of type 1 diabetes. Environmental impacts notwithstanding, genetic impacts on the age of onset are strong, as shown by single gene mutations that remove regulatory T cells leading to diabetes in the first week of life, and by the observation that people with adult-onset type 1 diabetes often do not have the high-risk genetic variants (e.g., *HLA* alleles) borne by people who develop the disease as children.

- **Study role of innate immunity in diabetes.**

Although analysis indicates that type 1 and type 2 diabetes do not share many genetic risk factors, research has demonstrated that multiple cytokine pathways are dysregulated and innate immunity may be important for beta cell loss and complications in both diseases. For example, the cytokine IL-1 and the opposing IL-1 receptor agonist (IL-1Ra) are being studied as a therapeutic in both type 1 and type 2 diabetes. In addition, IL-1 beta antagonists are also being actively studied in basic and clinical research. Research should be pursued to further examine this possibly shared disease pathway.

Animal Models/Translational Efforts from Pathogenesis to Therapy

New animal models of type 1 diabetes and autoimmunity are opening up research opportunities that were not possible before. In particular, mouse models with greater fidelity to human disease are becoming increasingly useful research tools for studying underlying mechanisms of disease and for testing new therapies. Researchers will soon be able to capitalize on the development and availability of these resources and related tools to expand fundamental understanding of the disease and to assist in translation of promising pre-clinical findings into treatments for patients and people at risk for type 1 diabetes.

Key Questions

- **Can higher fidelity mouse models of human disease be developed that will improve the ability to predict the efficacy of new therapies in patients?**
- **Can NOD mice (and/or higher fidelity mouse models of human disease) be used to:**
  - **Perform systematic screening of small molecules or other potential therapies for prevention or reversal of type 1 diabetes?**
  - **Identify environmental agents that precipitate or prevent type 1 diabetes?**
  - **Identify biomarkers in the blood that can monitor islet cell mass or autoimmunity?**
- **Can the function of human diabetes susceptibility or protective genes be effectively studied in mouse models?**
- **Can common pathogenic mechanisms be identified among different autoimmune diseases and in different disease models that may inform the search for new therapeutic targets and strategies?**

Future Directions

➤ **Rapidly translate new findings on disease pathogenesis in animal models into potential therapies.**

Although findings in NOD mice led to clinical trials testing insulin, anti-CD3, and other agents, it will be important to use animal models to move beyond these approaches to the next generation of immunomodulatory therapeutics. New targets should be identified and rapidly validated in animal models, and the mechanism of action determined to facilitate their translation to humans. New approaches, such as cellular therapy using stem cells, regulatory T cells, or modulation of novel cell targets (e.g., NK cells, NKT cells, B cells) may be modeled in animals and translated into the clinic.

➤ **Use animal models to identify and validate biomarkers of type 1 diabetes.**

Animal models may provide key insights into biomarkers for human type 1 diabetes. Future research to identify and validate translatable biomarkers could possibly facilitate the ability to predict disease and to measure the efficacy of therapeutic intervention.

➤ **Develop a higher fidelity mouse model of human disease that develops type 1 diabetes.**

A mouse that has a functional human immune system and that develops type 1 diabetes may provide an ideal

pre-clinical model for studying human type 1 diabetes without putting patients at risk. Ideally, such a model(s) would advance research into the pathogenesis of human disease, and would enable the identification of genes, environmental agents, and therapeutics that are directly relevant to people with type 1 diabetes. A current barrier to research progress is that many therapeutic agents being tested in the clinic are specific for humans and cannot be adequately tested in mice. Thus, such a mouse model would be an extremely valuable tool for evaluating these types of agents before they are tested in people. In addition, development of higher fidelity mouse models of human type 1 diabetes could provide a battery of models of type 1 diabetes that more closely reflect the genetic heterogeneity of humans. Having models of disease that can accommodate the natural diversity of the human population, rather than relying on the response of a single inbred strain of mice with a single set of diabetes susceptibility and resistance genes, would dramatically expand the ability to identify mechanisms of pathogenesis and surrogate markers of active/inactive diabetes that are uniquely human. Development of such sophisticated mouse models of human disease to support research in type 1 diabetes will be very challenging, and likely will require extensive genetic manipulations to replace species-specific molecular systems (e.g., cytokines, receptors, signaling molecules, adhesion, and trafficking) to create the appropriate environment for modeling the human disease process.

➤ **Develop *in silico* models for type 1 diabetes.**

There has been progress in developing *in silico* (computer) models for the study of multiple diseases, including type 2 diabetes. These *in silico* models permit hypotheses to be tested rapidly and help to inform the design of experiments to further test and validate hypotheses *in silico* and in biological systems. The development of *in silico* models for type 1 diabetes would greatly accelerate progress in understanding the pathogenesis of type 1 diabetes, as well as in identifying and modeling the potential of various therapeutics prior to pre-clinical testing in animal models and testing in clinical trials.

Beta Cell Function in Type 1 Diabetes: Autoimmune Attack and Prospects for Recovery

Although many questions remain, recent research findings about beta cells and autoimmunity are improving prospects for developing strategies to preserve and enhance beta cell function that may delay or prevent progression of type 1 diabetes. Efforts to understand fundamental beta cell biology, described in "The Beta Cell" chapter, will be key to developing these strategies as well.

Key Questions

- **What is the beta cell mass/function at onset of type 1 diabetes?**
- **How much residual beta cell mass/function is required for reversal after immunotherapy? Does it differ with different treatments?**
- **Can mechanisms that protect mouse cells from autoimmune destruction also protect human islets from autoimmune attack?**
- **Why is pancreas volume greatly reduced in people with type 1 diabetes? Does this reduction have an influence on disease parameters? Can it be used as a biomarker of disease development or potential for success in therapeutic intervention?**
- **Are there diabetes-susceptibility genetic variants that determine the ability of**

beta cells to resist autoimmune attack, or to regenerate or recover function once autoimmunity is controlled?

Future Directions

- **Develop metabolic tests to detect early signs of beta cell dysfunction.**

Additional metabolic tests that detect more subtle dysfunctions in genetically-defined at-risk populations than is currently possible could be used to determine whether the known autoantibodies precede metabolic disturbance or are the result of beta cell dysfunction. Metabolic changes could potentially also be used to improve disease onset prediction during the prodomal phase.

- **Examine the effect of insulin resistance on the development of type 1 diabetes.**

It has been hypothesized that some type 2 diabetes phenotypes, such as insulin resistance, can accelerate the onset of type 1 diabetes, at least in a subset of individuals. If true, this could be used to better predict risk and timing of onset, and could provide an explanation for the increased incidence of type 1 diabetes in puberty—a time period when insulin resistance is higher.

- **Identify genes and mechanisms that protect beta cells from autoimmune dysfunction and/or destruction, in animal models or in humans when possible.**

Beta cell biologists are developing an increasingly detailed understanding of the effects of inflammation, hyperglycemia, and other stressors on beta cell function, survival, and replication, especially in the context of type 2 diabetes. More detailed understanding is needed of how autoimmunity in type 1 diabetes affects the beta cell and whether opportunities exist to protect the beta cell from autoimmune damage. Part of the difficulty arises from the rapid timescale of autoimmune destruction and the inability of beta cell biologists to access the pancreas during this critical time frame. Therefore, better tools are needed to allow dissection of the effects of autoimmunity on the beta cell.

- **Define specific and sensitive surrogate markers of physical and/or functional beta cell recovery in response to immunotherapy and determine if beta cell mass can regenerate without reactivating autoimmunity.**

As noted previously, a growing body of evidence has suggested that researchers are getting closer to identifying a means to halt the autoimmune process underlying beta cell destruction. If this hope becomes reality, the benefits to the diagnosed patient are likely to be indirect at first (e.g., a reduction in daily exogenous insulin usage, production of some endogenous C-peptide which could help overall glucose control). The longer-term goal would be to stimulate beta cell regeneration with the halt of autoimmunity. Therefore, sensitive and specific markers are needed that reflect not only the degree of beta cell mass that exists at the time immune intervention occurs but, in addition, those that may reflect any recovery that beta cells see following attenuation of the autoimmune response. Indeed, researchers actively question to what degree beta cells are capable of self-regeneration and, beyond this, whether such regeneration would once again initiate or rekindle a destructive autoimmune response.

Immune Mechanisms of Pancreatic Pathology

To understand the pathogenesis of type 1 diabetes and the autoimmune destruction of pancreatic beta cells, better basic information and research tools or systems for analysis are needed. For example, questions remain as to how effector or pathogenic autoimmune T cells are created or maintained, and through what basic mechanisms they are controlled by other cells, including the regulatory T cells that are known to dampen pathogenic responses. It is also important to understand the role of B cells as autoantibody producers, as well as antigen presenting cells, in the pathology of type 1 diabetes.

Key Questions

- **How diverse are the T and B cell responses to individual diabetogenic antigens, and how can the dominant effect of major histocompatibility complex (MHC) sequence on diabetes susceptibility be explained?**
- **What are the respective roles of $CD4^+$ and $CD8^+$ T cells, as well as other immune cell subsets (e.g., B cells, NK cells, dendritic cells, and mast cells), in pathogenesis?**
- **What is the role of regulatory cell populations in diabetes pathogenesis or protection?**
- **What is the relationship between autoimmunity and inflammation in type 1 diabetes, and what are the roles of other organs such as gut, liver, fat, or others?**
- **What underlies the variability of attack on different islets within the same pancreas, and can that understanding be used to interdict the disease process?**

Future Directions

➤ **Identify the range of tolerance mechanisms defective in type 1 diabetes models and patients (e.g., genetic polymorphisms in immune system genes) and delineate precisely where the cellular and molecular defects lie.**

Over the last decade, the list of immune system components in people with type 1 diabetes that could be defined as defective, due to their inability to impart tolerance to beta cells, has grown in number. At the same time, as noted in the "Genetic Basis of Type 1 Diabetes, Type 2 Diabetes, Obesity, and Their Complications" chapter, the number of genetic loci considered to modulate susceptibility to type 1 diabetes has seen marked expansion—nearly 50 genes or genetic regions have been identified—the majority of which have potential to be involved in the immune system. As a result, the research community needs to establish a blueprint for the nature and order of these immune system defects (i.e., which ones could be considered primary, and which ones represent downstream effects of earlier defects) and determine how genetic differences impart these immune system defects. Pursuing such research has the potential to better explain the pathogenesis of type 1 diabetes, lead to improved disease prediction, and allow for the development of better targeted therapeutics.

➤ **Define how auto-inflammatory infiltrates and beta cells communicate with each other in controlling type 1 diabetes progression.**

A better understanding of autoimmunity-relevant changes within the beta cells, such as upregulation of molecules that present antigens to cytotoxic T cells (MHC class I molecules), or induction of inflammatory antigen processing machinery, could provide new therapeutic options. Research should also be pursued to understand how cross-talk between beta cells and cells of the innate and adaptive immune systems impinges on the progression and treatment of diabetes. For example, inflammatory and autoimmune processes (including local cytokine production) could directly alter beta cell function, which could in turn activate more inflammation or autoimmunity. This understanding could form the basis for new combination therapies. If beta cell regeneration is feasible in humans, it will be important to define the physiological stimuli that elicit beta cell regeneration in response to acute beta cell ablation and to establish how chronic autoimmune inflammation interferes with the induction and/or effector function of these stimuli. If in the end it is a kinetic issue—i.e., more beta cells are lost than are being regenerated—therapies can be developed to target that issue.

- **Understand the repertoires of responding lymphocytes, including T cells and B cells.**

For many years, scientists believed that the immune system cells infiltrating the pancreatic islets and rendering the damage that results in type 1 diabetes not only saw multiple antigenic targets, but were themselves diverse in their expression of certain surface markers (i.e., in the case of T cells, their T cell receptor molecules). That hypothesis has changed recently, as data from animal models, as well as very limited information from human pancreata, suggest that the repertoire of T cells entering the islet may be much more focused. This concept, however, remains preliminary in nature, as its proof will rely on the availability of pancreatic tissue specimens obtained from individuals collected throughout the natural history of type 1 diabetes (i.e., before disease onset, at onset, and continuing until long after disease onset). Addressing this question could dramatically improve understanding of why type 1 diabetes develops and could also help guide the development of a highly specific means to prevent the disease.

- **Identify the range of regulatory cell populations potentially defective in type 1 diabetes and learn which regulatory populations—defective or not—provide good therapeutic opportunities.**

A complete catalog of the cells involved in the body's misguided attack on its own beta cells would likely accelerate opportunities for therapy—any important player could provide insight and opportunity for a successful intervention. Research should be pursued to define the roles of T cells, B cells, NK cells, dendritic cells, mast cells, and others in type 1 diabetes pathogenesis. A large body of literature also suggests an important role for cells that, based on their properties, would be considered regulatory. Indeed, a greater understanding of regulatory T cell populations could provide insight into how to manipulate "master switches" in the immune system and soften or mute the attack on pancreatic beta cells. In addition, studies should define methods to isolate and expand these cells outside of the body so that they could be tested for safety and therapeutic benefit upon re-introduction into a person with type 1 diabetes, alone or in combination with novel or existing drugs.

- **Define which pathways are shared by different autoimmune diseases and which are disease-specific.**

One of the major recognitions in type 1 diabetes research over the past decade was the discovery that a small but appreciable percentage (approximately 5 percent) of people with type 1 diabetes also has another autoimmune disorder, celiac disease (10). The finding of autoimmune disease in association with type 1 diabetes is not new. For example, it has been known for decades that people with the disease are also at increased risk for autoimmune thyroid disease. These situations nonetheless form remarkable opportunities to define disease-specific mechanisms (both genetic and immunologic) by performing studies in people having only one disease, versus those who have disorders in combination with each other. This information will help the field make informed decisions about the applicability to type 1 diabetes of particular immunotherapies that are being tested or used in more prevalent autoimmune diseases.

- **Extend and preserve existing pancreas repositories and data banks, which are critical for direct examination of pancreatic pathology.**

The value of these resources for type 1 diabetes research is clear. For example, pancreatic tissues could be probed for viruses which could then be candidates for triggering infections and studied using the resources of samples from human populations at risk for type 1 diabetes. In addition, the pancreatic and draining lymph node samples banks could be used to define number and specificity of autoreactive T cells in human and mouse islets and to understand their heterogeneity (in terms of T cell receptor usage and antigen recognition). Information gained from pancreas pathology could also possibly be used to help recreate the human islet using stem cells.

IMPORTANCE OF RESEARCH GOALS AND STRATEGIES: HOW TRANSLATING RESEARCH OUTCOMES MAY LEAD TO IMPROVEMENTS IN HEALTH

Overcoming type 1 diabetes will rely on multiple and diverse research efforts, from basic research to clinical trials and epidemiological studies. Bolstering coordination among clinical researchers, government agencies, and industry should aid the flow of candidate interventions into clinical testing—such as that seen in the NIH-supported Type 1 Diabetes TrialNet—and facilitate rapid redirection or addition of efforts in light of new scientific findings. These efforts, in turn, will rely on greater understanding of how type 1 diabetes develops, both through studies in people to uncover genetic, environmental, and other contributing factors, and through improved animal models of the disease. Animal models will also play a crucial role in the identification and testing of interventions for their potential utility in combating type 1 diabetes and its complications. Biomarkers that are accurate predictors of early pathogenesis in people will be critically important for enabling effective disease prevention. Similarly, biomarkers of autoimmune pathogenesis or beta cell destruction or recovery will be needed to monitor the efficacy of interventions designed to reverse the disease or its complications after onset. Results from fundamental studies of normal immune mechanisms and autoimmunity could help explain the onset, progression, and heterogeneity of type 1 diabetes, and potentially point the way to new approaches to prevent, reverse, or treat the disease.