ADA Outstanding Scientific Achievement Lecture 2004

Thirty Years of Investigating the Autoimmune Basis for Type 1 Diabetes

Why Can't We Prevent or Reverse This Disease?

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Thirty years ago, a convergence of investigational observations lead to the now widely accepted notion that type 1 diabetes results from an autoimmune destruction of insulin-producing β-cells in subjects genetically predisposed to the disease. Improvements in understanding of the natural history of type 1 diabetes, the biochemical identification of autoantigens, the discovery of spontaneous animal models for the disease, the availability of immune-modulating agents, and other important facets, including disease prediction, drove an early sense of optimism that the prevention of type 1 diabetes was possible and, in some research circles, that ability was thought to be within a not-to-distant reach. Unfortunately, those early expectations proved overly optimistic, and despite the aforementioned knowledge gains, the generation of improved investigational tools, the identification of methods to prevent the disease in animal models, and the formation of very large disease prevention trials, a means to prevent type 1 diabetes in humans continues to remain elusive. Believing in the concept of "informative failures" (a.k.a., wise people learn from their mistakes), this lecture reviews the knowledge base collected over this time period and, when combined with an analysis of those research experiences, sets forth a proposal for future investigations that will, hopefully, turn discoveries into a means for the prevention or reversal of type 1 diabetes. *Diabetes* 54:1253–1263, 2005

I'll begin this lecture the same way that I will end it: by conveying a heartfelt word of thanks to the many people who deserve recognition on my acceptance of this award, as well as to recognize those who have contributed to the science that stands behind it. At the end of the talk, I will recognize a few of these special people. But for now, thanks are extended to my colleagues who selected me for this year's award. A great deal of thanks is also extended to the American Diabetes Association (ADA) for the impact of this organization on my entire career. The ADA has always been there for me, for us who care about diabetes, and for those with the disease. Upon hearing of my receipt of this award, I was advised that the best award lectures of the past have told a story. So, in taking that advice, today I will tell a story; but, if it were to be bound into a book, it would not easily fit into a particular subject category on the shelf at your local bookstore. Parts of the story would suggest that it belongs in the history section, yet in other ways, this story would be a drama, a comedy, a tragedy, a lesson on geography, a primer on public health and nutrition, and, perhaps most of all, an unsolved mystery.

Where would one begin the story? In telling a story about type 1 diabetes, one could begin in many different places or situations. One could begin at the first written clinical descriptions of diabetes, around 1500 BC. One could envision beginning a story on type 1 diabetes at the time of Banting and Best, in the 1920s. That Nobel Prize–winning event involving the isolation of insulin is surely noteworthy for what it did for treating individuals with the disorder. A case could also be made to start with the new-found crisis that arose in the 1940s with the advent of diabetes-associated complications—an unfortunate facet that remains today and has become a focus of an immense degree of investigation.

However, for this presentation, I have decided to begin by recognizing a different milestone in the history of type 1 diabetes. Specifically, I've elected to use a 30-year timeline for this presentation, a fact that I admit up front predates my personal research experiences in the field by nearly 10 years. In 1974, we saw a convergence of a series of studies that collectively had a dramatic influence on what, at the time and for a number of years thereafter, was commonly referred to as "insulin-requiring" or "juvenile-onset" diabetes. Indeed, in 1974, investigators had nearly a decade to digest the studies published by Dr. Gepts in the journal *Diabetes* regarding the anatomy of the pancreas (Fig. 1) from individuals with type 1 diabetes (1). These studies led to the widespread appreciation of the immune

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ADA, American Diabetes Association; ICA, islet cell cytoplasmic autoantibody; NIH, National Institutes of Health.

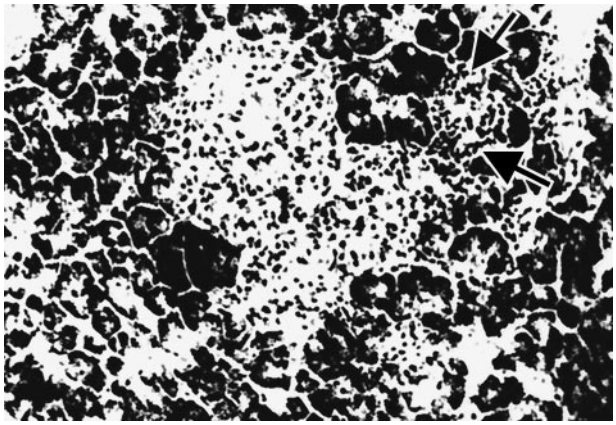

FIG. 1. Islet of Langerhans in human type 1 diabetes. As adapted from 1, this represents the inflammatory infiltrate from a 2-year, 10-month-old male type 1 diabetic patient with a disease duration of 60 days. Hemalum erythrosine saffron. Magnification ×130.

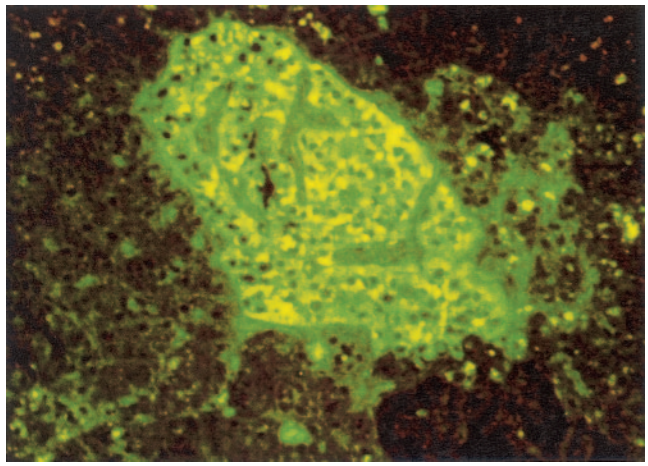

FIG. 2. The ICA. Serum from patients with or at increased risk for type 1 diabetes often possess antibodies that react with the cytoplasm of pancreatic islet cells. These reactivities are determined by indirect immunofluorescence testing of serum samples with blood group O human pancreas. The titer of these autoantibodies can vary dramatically and are quantified in JDF (Juvenile Diabetes Foundation) units based on comparison to a WHO (World Health Organization) standard. The antigens to which ICA react are multiple and include GAD, ICA512, glycosphingolipid, and other undefined molecules.

infiltrate of the pancreatic islets commonly referred to today as insulitis. The pathologic significance of this infiltrate at the time was unclear; only 68% of pancreatic specimens from recent-onset type 1 diabetic patients showed insulitis, and of those, an even smaller percentage showed marked intraislet inflammation. The human insulitis lesion remains a mystery of sorts until this day, but nonetheless, that study showed the potential importance of the immune system in the development of type 1 diabetes. In the early 1970s, we also began to appreciate the "geneticist's nightmare" of type 1 diabetes (2), with studies of Dr. Nerup and others indicating that immune system molecules associated with the human leukocyte or HLA antigens influenced the risk for type 1 diabetes and, when combined with investigations supporting cellular hypersensitivity to pancreatic antigens, laid the groundwork for a role for a self-directed immune response in individuals developing the disease (3,4).

The milestone that begins this story. At the same time, investigators analyzing serum samples from subjects with polyendocrine autoimmunity, including those with type 1 diabetes, noted that such individuals possessed antibodies that reacted with pancreatic islet cells (5). Despite early debate as to who deserved credit for the finding of autoantibodies in type 1 diabetes (as similar studies were being performed in a like time frame in other parts of Europe [6]), it was this publication in 1974 describing islet cell cytoplasmic autoantibodies (ICAs) that many recognize as establishing the date for which an autoimmune pathogenesis for type 1 diabetes began. From this publication, Gian Franco Bottazzo, a previous Banting award recipient, helped move forward a field of investigation in type 1 diabetes that changed the way we thought about this disease. Quite remarkably, for much of the past 30 years, the ICA test remained a cornerstone of type 1 diabetes research. It proved quite a useful tool in the diagnosis of type 1 diabetes, as well as in the identification of individuals at increased risk for the development of the disease. Without it, trials for disease prevention would not have been possible until a much more recent date. However, with the passage of time, this test has become less popular and the actual reaction unfamiliar to many, so much so that I have elected to include it in this presentation (Fig. 2).

Early predictions/resulting realities. With this finding of autoantibodies, we entered a decade in which research investigating the immunology, genetics, and pathogenesis of type 1 diabetes saw a dramatic increase. Given the writings of that period, I have formulated what I believe was a general consensus on the pathogenesis of type 1 diabetes for the subsequent decade, as well as the prospects for a cure for this disorder (Table 1). As previously mentioned, it was thought that the genetics of type 1 diabetes would represent a relatively simple genetic susceptibility, with HLA providing the predominant, if not near-exclusive genetic risk for the disease. With studies supporting the diagnostic value of autoantibody markers, and early studies showing their ability to identify individuals at risk for the disease, it was the hoped that predictive tests would soon be available for widespread use. Many believed type 1 diabetes was caused by a molecular mimicry event, induced by sequence similarity between diabetogenic viruses and a β-cell protein. Immunosuppressive agents, so valuable for their efficacy in organ transplantation, raised hopes for their ability to reverse type 1 diabetes in those with the disorder. It was also during this time period that two spontaneous animal models for type 1 diabetes were introduced, the biobreeding (or BB) rat and the nonobese diabetic (NOD) mouse. Optimistic investigators thought that with the availability of animal models, all one needed to do was to identify an agent capable of disease prevention and it could be rapidly translated to humans. In addition, through identification of genes responsible for type 1 diabetes in these animal models, similar genetic regions in humans could soon be identified, leading to major improvements in the understanding of the genetics of type 1 diabetes in humans. Studies of animal models of the disease involving adoptive transfer pointed to a disorder that was caused by T-cells, whereas B-lymphocytes or autoantibodies were seen as relatively insignificant in terms of disease pathogenesis. Finally, it was also during this time period that the notion for islet cell trans-

TABLE 1
Early predictions of the autoimmune era on the pathogenesis and prospects for prevention of type 1 diabetes (ca. 1974–1986) and their modern day (ca. 2004) reality

	Prediction	Reality
Genetics	Relatively simple genetic susceptibility (i.e., mostly HLA)	Very heterogeneous (i.e., >20 loci), population dependent
Autoantibodies	Predictive tests "soon"; potentially simple and widespread	Numerous "practical" hurdles offset acceptance and use
Etiopathogenesis	Likely caused by viral infection with or without molecular mimicry	Unknown; viral mimicry out of favor
Intervention therapies	Immunosuppressive agents to reverse disease	Multiple studies, including immunosuppression based, have failed to reverse or prevent disease
Animal models	Identify agents for disease prevention, aid in human genetics	Thus far, limited aid to identification of therapeutic agents or genes in humans
Immune effectors	T-cell mediated; B-cells and autoantibodies insignificant	Unknown; B-cells and autoantibodies may influence disease
Islet cell transplantation	"Close"	"Closer"

plantation representing a cure for the disease became the subject of much scientific and public discussion, and that we were close to achieving that goal.

H.L. Mencken said, "For every problem there is an answer that is clear, simple, and wrong." Call it "Monday morning quarterbacking" or proving the notion that "hindsight is 20/20," looking back on this time period, we can see (Table 1) that a number of these assumptions were, unfortunately, incorrect—but the reasons for this vary. The early prediction that type 1 diabetes would involve relatively simple genetic susceptibility involving the HLA region with near exclusiveness has certainly proven overly optimistic, as we now know that many more loci are associated with genetic susceptibility to type 1 diabetes (7), shown both in terms of their loci (Fig. 3 and Table 2) or that of candidate genes (Table 3) (8). In terms of autoantibody markers, the notion that predictive tests would soon be available did become true. However, the translation of autoantibody testing beyond the research arena and into public health practices has been slow (9). While the notion that type 1 diabetes results from viral mimicry still exists today, that thought has been relegated to a relatively small group of investigators, as years of attempts to identify areas of amino acid similarity between β-cell self-antigens and environmental agents have failed to identify any of pathogenic significance (10). After years of study, immunosuppressive agents to reverse disease progression proved effective for a few, but ineffective over the long run and of questionable safety (11). In terms of the impact of animal models, without question, they have led to some of the most exciting discoveries in type 1 research. However, they have also proved to be a disappointment in terms of providing simple solutions to the issues of genetics of type 1 diabetes and disease prevention (12). The notion that type 1 diabetes is caused by T-cells alone has come under recent question, as studies of B-lymphocytes now indicate that they do play important roles in β-cell destruction (13). Finally, islet cell transplantation has for much of the past 30 years remained an elusive goal. With the recent Edmonton trial experience, it appears that we are closer, but not quite there yet (14,15). Hence, the notion for the title of my presentation.

Thirty years of investigating the autoimmune basis for type 1 diabetes: why can't we prevent or reverse this disease? The question most often asked of me by individuals with type 1 diabetes or their family members is "when" are we going to prevent or reverse the disease. The second most common question is "why." Why, despite 30 years of understanding an autoimmune nature of this disease, haven't we been able to prevent it? As all good puzzle solvers know, a key step involves finding the corner pieces. In our effort to solve the puzzle as quickly as possible, I believe we were unsuccessful in finding the four corners.

Puzzle piece one: what is "it"? The first of these was a failure to understand and appreciate what actually causes type 1 diabetes. Much of the problem that has plagued our community of scientists in finding the piece of the puzzle on "what causes type 1 diabetes" has been a failure to ade-

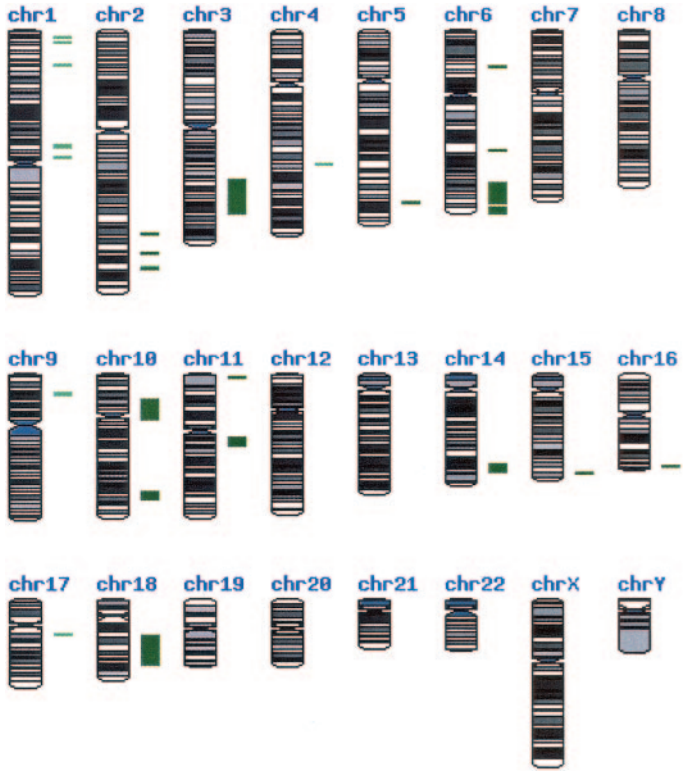

FIG. 3. The genetics of human type 1 diabetes. As adapted from 8, multiple loci have been implicated with influencing the susceptibility or resistance to type 1 diabetes. Shown are illustrations of human chromosomes 1–22 and X/Y, with loci having published support for an association with type 1 diabetes (green bars).

TABLE 2
The genetics of human type 1 diabetes

Loci	Region
HMO_Idd9.1	1p34.3
HMO_Idd9.2	1p36.21–36.22
HMO_Idd9.3	1p36.22–46.23
HMO_Idd10	1p12
HMO_Idd18	1p13.2–13.3
Unnamed chromosome 1	1q42.2
IDDM7	2q32.2
IDDM12	2q33.3
HMO_Idd5.2	2q35–36.1
IDDM13	2q36.1
IDDM9	D3S1303
HMO_Idd3	4q27.28.1
IDDM18	5q31.2–35.1
IDDM1	6p21.31
IDDM15	6q22.1
IDDM5	6q23.3–25.3
IDDM8	6q26–27
HMO_Idd9.4	9p21.3–22.1
IDDM10	10p11-q11
IDDM17	10q25.1–26.11
IDDM2	11p15.5
IDDM4	11q12.2–13.1
IDDM11	14q31.3–32.13
IDDM3	15q26.1–26.2
Unnamed chromosome 16	16q24.1
HMO_Idd4	17q12–21.1
IDDM16	18q12.3–22.1

Candidate loci associated with influencing the risk for type 1 diabetes. Arranged in approximate chromosomal order (i.e., chromosome 1 through Y) and adapted from 8.

TABLE 3
The genetics of human type 1 diabetes

ACE	ICAM1	NFKB1
ADAM33	ICOSL	NOS3
AGT	IFNG	PSMB8 in IDDM1
AIRE	IGF1	PTPN22
ALDRL2	IL10	PTPRC
APOE	IL12A in IDDM9	REG1A
AR	IL12B in IDDM18	PADI4
B2M	IL13	PDCD1LG1
B7	IL18	PPARG
BAT2	IL18BP	REG1B
BCL2 in IDDM6	IL1A	SLAMF1
CASP10	IL1RN	SLC11A1 in HMO_Idd5.2
CASP7 in IDDM17	IL21R	TAP2 in IDDM1
CASP8	IL2RA	TCF7
CBLB	IL2RB	TGFB1
CCR5	IL2RG	TLR1
CD14	IL4	TLR10
CD28 in IDDM12	IL4R	TLR2
CD4	IL6	TLR3
CD48	IL8	TLR4
CD80	INS in IDDM2	TLR6
CD86	IRF1	TLR7
CFLAR	IRS1	TLR8
CP in IDDM9	KCNJ11	TLR9
CRP	KIR2DS2	TNF
CTLA4 in IDDM12	KIR3DL1	TNFRSF11A in IDDM6
CXCL12 in IDDM10	KLRC4	TNFRSF18
CYP27B1	LAG3	TNFRSF1A
ERVK2	LCK in HMO_Idd9.1	TNFSF11
ERVK3	LCT	TNFSF5
FABP2	LRP5 in IDDM4	TNFSF6
GAD2 in IDDM10	MBL2	TNFSF9
GCK	MHC2TA	TREM2
GLP1R	MICA	TYROBP
HSD11B2	MRC1	VDR
HSPG2	NAT2	
IAN4L1	NEUROD1	

Candidate genes associated with influencing the risk for type 1 diabetes. Arranged in alphabetical order. Non–HLA DR/DQ candidate genes associated with type 1 diabetes are noted. Adapted from 8.

quately address the eternal argument of how much of the disorder results from genetics versus the contribution of the environment.

One notion often used to suggest an environmental component for type 1 diabetes relates to differences in the frequency of the disorder across many populations (16). Indeed, among different regions of the world, we can see variances of up to 500-fold in the frequency of this disease—a facet that, when combined with the rapid rise in disease frequency in the last few decades, points to environment as a major contributor (17). If one believes in the role for environment in this disease, the obvious question is "what is it?" Over the past 30 years, we have seen no shortage of candidates for the title of *the* environmental agent in type 1 diabetes. Early candidates were principally thought to be viruses, a fact that in part may have been held over from what was once considered an acute pathogenic nature for this disease (18). Studies in the early- to mid-1980s pointed toward the frequency or duration of breast-feeding (19). Other investigators have viewed the consumption of cow's milk or infant formula as the cause of type 1 diabetes (20,21). The practice of infant immunization came under much public scrutiny for its potential to influence autoimmune diseases such as type 1 diabetes (22). The degree of a society's cleanliness and improvements in it over time has lead to the development of a "hygiene hypothesis " that portends that improvements in health care delivery and sanitation have lead to the rise in autoimmune disorders including type 1 diabetes (23,24). Last fall, studies reported that the timing of introducing cereals into infant diets influenced the development of anti–β-cell autoimmunity (25–27). Complicating matters even more, recent studies have implicated stressful lifestyles and dietary practices such as the consumption of artificial sweeteners, caffeine, and even smoked fish. Hence, many candidates, but no obvious answers.

Unfortunately, much of the literature surrounding each of these environmental candidates has been extremely conflicting. One of but many examples was our study (28), which did not confirm the findings of others (29) suggesting that 100% of subjects with type 1 diabetes had antibodies against the molecule BSA (bovine serum albumin), thus casting some degree of doubt on the role for this molecule in the disease. This has been, for the subject of environmental agents in type 1 diabetes, an unfortunate and recurrent theme: nonreproducibility across studies of various populations.

Many of us were taught a basic principle in algebra to first solve one side of the equation, and with it, we could solve the other side. So, if in our genetics versus environment equation the side of environment seems too difficult, then perhaps turning to genetics might be more fruitful. However, as summarized in the accompanying illustrations (Fig. 3 and Tables 2 and 3), like our understanding of environment, studies of genetics in human type 1 diabetes have also proven remarkably difficult. As of today, over two dozen different loci and 100 candidate genes, albeit to

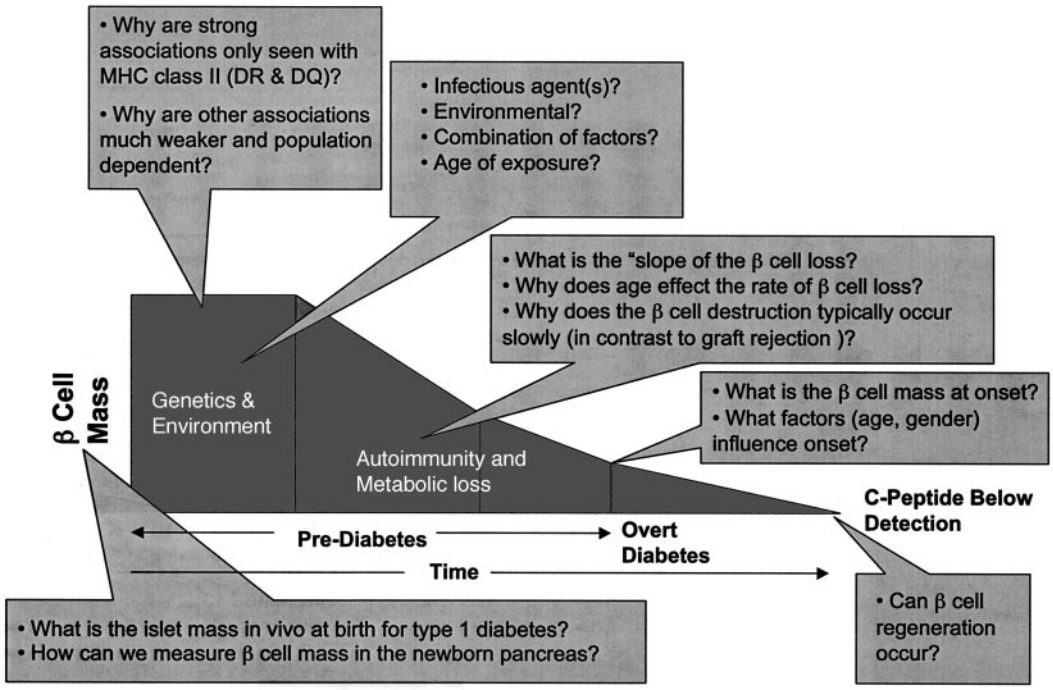

FIG. 4. Shortcomings in our understanding of the natural history of type 1 diabetes. Building from the widely popular and referenced model (31), this illustration demonstrates a series of shortcomings and outstanding questions regarding our knowledge of the natural history and pathogenesis of type 1 diabetes in humans.

markedly different degrees of certainty, have been ascribed to influence susceptibility to type 1 diabetes, and despite massive amounts of scientific effort, only a limited number of these loci have been ascribed to particular genes; even in the cases involving gene identification, the role for most if not all in the actual pathogenesis of the disease remains a mystery (30). Hence, both sides of the equation to solve the puzzle piece of "what causes type 1 diabetes " have proven remarkably elusive.

Puzzle piece two: what is the natural history of type 1 diabetes? The next corner piece of the puzzle that has not been gathered to impart our ability to prevent or reverse type 1 diabetes is that of a failure to adequately understand the natural history of this disease. Without question, the most popular model for the natural history of type 1 diabetes for a period nearing 20 years has been the model proposed in 1986 by George Eisenbarth (31). In this model, a person born with genetic susceptibility to type 1 diabetes encounters a disease-inciting environmental agent, a process that elicits an autoimmune reaction that remains metabolically quiet, but one that can be identified by autoantibodies. Only in the latter stages of the disease, when a vast majority of β-cells are destroyed, does one encounter glucose intolerance, overt diabetes, and the eventual absolute loss in β-cells and C-peptide function.

While models such as this are crucial for scientific testing, and this one in particular deserves credit for the way it drove a generation of type 1 diabetes research, problems arise when too many facets are recognized as dogma. For this lecture, I consulted with Dr. David Harlan in an effort to summarize many of our knowledge voids as they relate to the natural history of type 1 diabetes in humans (Fig. 4). The first void actually begins at birth, that being the notion that an individual is thought to be born with at or near 100% of their β-cells and that those cells are "normal." Because of practical difficulties, this facet cannot be taken with certainty because, at this time, we still cannot easily or accurately determine islet cell mass in vivo or ex vivo in humans, and literature does exist suggesting variance (27). In the next phase, we look at genetic susceptibility. While we know there is a strong association with MHC (major histocompatibility complex) class II molecules, other associations have proven themselves much weaker and population dependent, including the insulin VNTR (variable number tandem repeat) and CD152 molecules (30). The next phase of the model is that involving the inciting event. To date, we still do not know what infectious agents are involved, how they act, or when they act (32). The next period, that being of the silent β-cell loss, also has limitations in certainty. The first is that it is unclear what the slope of that β-cell loss is; specifically, is the loss linear or does it contain periods of stepwise remission (33)? We are also unclear of what underlies the effects of age, sex, and genetics on this slope or when the process begins. Finally, and this remains a key puzzle for immunologists in particular, we must question why β-cell destruction typically occurs slowly over a period of perhaps months to years to decades in contrast to graft rejection or many other immune responses, where the process can occur in periods of hours to days (30)? We then enter the phase of the model questioning β-cell mass at disease onset. Common thought portends that the symptoms of type 1 diabetes usually begin when 90–95% of the β-cells are destroyed (34). The aforementioned studies of Gepts, in part, support this notion (1). However, metabolic evidence supporting this fact has recently come under question, specifically indicating that the insulin secretion rates in response to mixed meal testing at disease onset are perhaps only one-half of those seen in healthy control subjects (35). Finally, in the period referred to as brittle

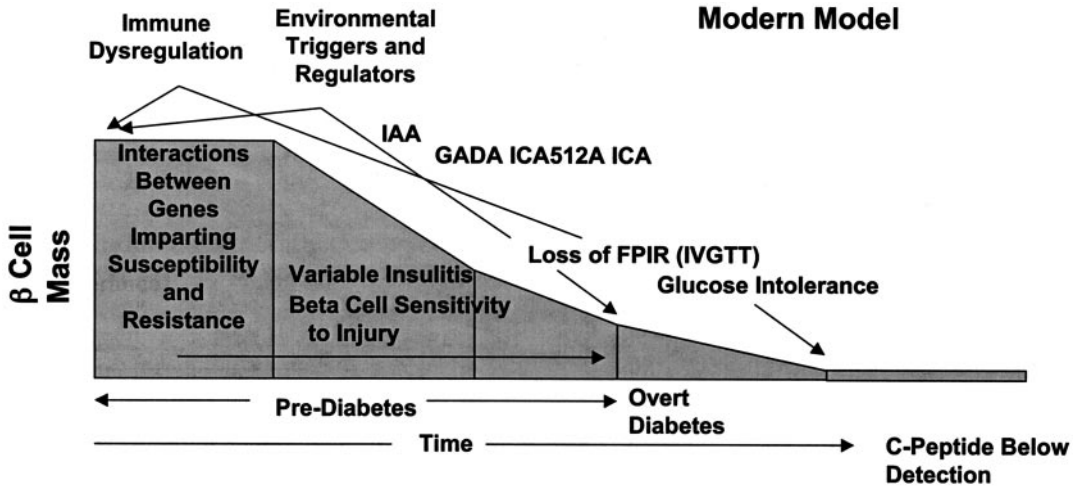

FIG. 5. A more modern model of the natural history of type 1 diabetes. Adapted from 37, this model includes many new facets of our understanding of type 1 diabetes not available in 1986 (31). While as indicated in Fig. 4, many knowledge gaps exist, significant evidence suggests that factors such as genetics and environment do influence the natural history of type 1 diabetes throughout the pre-diabetic period and are not limited to birth or single environmental triggers. Similarly, the influence of immune dysregulation also likely influences the type 1 diabetic patient throughout their lifetime. In terms of autoantibody formation, for many patients, the first autoantibodies observed are those of insulin autoantibodies (IAA), followed by GAD autoantibodies (GADA), ICA512 autoantibodies (ICA512A), and ICA. With continued β-cell loss, symptomatic onset occurs (i.e., over diabetes). Of recent question is whether a small amount of residual β-cell mass may in fact exist for periods of years to decades after diabetes onset, although its detection through C-peptide production would be below detection.

diabetes, we have also been under the assumption that months to years after the onset of symptoms, all islets are lost. However, once again new data are now bringing into question whether this factor is in itself true and whether β-cell regeneration can occur (36).

Hence, along with George Eisenbarth, we published an updated model (Fig. 5) that took into account some of these factors and proposed this as how the natural history of type 1 diabetes may develop, as well as highlighted a few of the major differences that now should be considered (37). Among them, rather than referring to simple genetic predisposition to type 1 diabetes, it would now be more accurate to note interactions between genes imparting genetic susceptibility as well as resistance to the disease. Furthermore, rather than implying genetic predisposition acts only in the period before autoimmune induction, one should consider that these genes actually influence the susceptibility and resistance to type 1 diabetes throughout the entire pre-diabetic period. In terms of environmental agents, rather than consider an individual precipitating environmental event, it may be more accurate to consider that environmental triggers and regulators of disease development act throughout the period before the onset of diabetes, perhaps from birth to the symptomatic onset of the disease. It may be that rather than the single occasion of having a random encounter with a virus, for example, it is the quantitative or qualitative impact of environment that plays a role in the development of type 1 diabetes (37). Another new facet to consider is that of immune dysregulation. Indeed, it is likely that immune deregulation impacts an individual from their earliest days of immune system activity to the period of onset of diabetes. The most recent evidence suggests that compromises in proper regulation of self-reactive and -protective immune responses may be the result of abnormal activities of a population of T-cells known as regulatory T-cells, which are distinguished by their expression of the CD25 molecule (38). In terms of humoral immune markers, rather than consider ICAs being the primary humoral immune response, the majority of studies thus far has suggested that it is antibodies against the insulin molecule itself that represent the first antibody that is recognized in the pathogenesis of type 1 diabetes (39,40). Another aspect that is worth reconsidering is the issue of slope of β-cell loss. It is likely that the slope will vary dramatically based on the degree and type of insulitis, the susceptibility of β-cells to destruction, and the capacity of those β-cells for repair and regeneration (41).

Before leaving the puzzle piece of the natural history of type 1 diabetes, there is one subfacet of it that must also be discussed as part of this presentation. That being a lack of understanding, in humans, of the role for the immune responses underlying β-cell destruction in general but, in particular, that of the cellular immune response to β-cell antigens (42). Rather than trying to use a series of figures and data to summarize this point, I believe the history of this aspect would be described through a series of adjectives—conflicting, confounding, confusing, contentious, and often conforming—all of this with the overriding difficulty that nearly all of our assessments involve investigations of mechanisms far from the site of chronic inflammation, the pancreas, but rather in the peripheral blood. One can only wonder what artifacts have been generated by such analyses over the years, including my own efforts in the area (43,44). Hope does however exist for newer technologies that have the capacity for detection of rare events in cellular immunity and that are consistent with the current understanding of the molecular events associated with recognition of antigen (45,46).

Puzzle piece three: practical and cost-effective means for prediction. The third piece to the puzzle of why I believe we have not been able to prevent this disease revolves around the issue of methods that can be used to predict the disease and how this can be done in a practical manner. The presentation of this puzzle piece may seem conflicting, as I have already mentioned that our ability to

TABLE 4
Potential "miscalculations" or unforseen barriers to practical type 1 diabetes prediction

Too rare disease incidence (1 in 300) to garner public health care attention for short term benefit of intervention
Costs for screening = ~$72,300 per case identified
Ethics of screening (what if we can't prevent the disorder?)
Issues of patient privacy
Difficulty in some autoantibody assay standardizations
Who will pay for screening?
When do we screen and how often?

use autoantibodies to predict future cases of the disease represents one of the major success stories of type 1 diabetes research in the last 30 years (47). Simply put, we *can* identify individuals both in the general population as well as relatives who have an increased risk for developing type 1 diabetes.

For this purpose, we have moved a long way from the ICA test into a modern era in which we routinely use multiple autoantibodies against defined autoantigens. These autoantibodies are often referred to as biochemical autoantibodies that are both platform based, show excellent quality assurance and quality control standards, and that have shown exceptional diagnostic as well as predictive value for type 1 diabetes. One of the major efforts in the research community to validate this notion came from our studies at the University of Florida on disease prediction, efforts that eventually were incorporated into the screening protocol for the NIH DPT-1 (National Institutes of Health Diabetes Prevention Trial–Type 1) (48). However, it is with disappointment that I discuss this ability, as much of my 21-year career in type 1 diabetes research was directed at efforts to derive a means to predict future cases of type 1 diabetes. In some ways, I would consider this both a success as well as a failure.

In short, when we as a research community began attacking this goal, the adage attributed to Ralph Waldo Emerson, indicating that "if we built a better mousetrap, the world would beat a path to our door step," appeared fitting. It was with much enthusiasm that this goal was pursued, an international organization even emerged in part to meet this goal: the Immunology of Diabetes Workshops (now known as the Immunology of Diabetes Society). However, there were a few key concepts that were not fully appreciated in efforts toward disease prediction. It may now be that the better piece of advice came from Yogi Berra: "Before you build a better mousetrap, make sure you have some mice out there."

Among the first shortcomings were the financial barriers. For this description, let me outlay a model for disease development. If we can assume that in the U.S. the incidence of type 1 diabetes is approximately 1 in 300 by the age of 20, we would find a disorder that would be considered of moderate but not high incidence. If I were to utilize the financial figures for screening published a few years ago by Dr. Ollie Simell, one would assume that the cost to screen for diabetes would be in the range of $241–788 per child (49). To simply take the risk associated with screening and multiply it by the low end of that cost estimate, one would come up with a figure of approximately $73,000 per case identified. Let me remind you that this would be a low-end cost. Simply put, I ask the same question of the type 1 diabetes community as I have asked of others: who is going to pay for this cost? In personal experience in meeting with various state health department directors, these costs do not meet actuarially analyses for attempts aimed at screening in combination with the prevention of the disease. Apart from costs, there are many many other limitations that stand in the way of practical disease prediction (Table 4). Among them are desirability (i.e., do individuals really want such information?), ethics (i.e., should we screen in the absence of a known method to prevent type 1 diabetes?), privacy (i.e., how will such information be handled), and practicality (i.e., who will do this screening?).

Puzzle piece four: overequating mice with man. Earlier in my lecture, I mentioned the notion that early optimism said if we could identify a method for preventing type 1 diabetes in the animal models of the disease, then this would lead to the development of a means to prevent the disease in humans. In 1994, I coauthored a study (50) wherein we referenced ~70 methods that could be used to prevent type 1 diabetes in NOD mice. By our publication of a related article in 1998, that list had expanded to 125 methods for preventing disease in NOD mice (51). Earlier this year, upon an invitation in Cambridge to debate the use of this model in diabetes, the most recent tally showed there were now 192 ways of preventing diabetes in NOD mice (52). Hence, the question has now become not can we find a way to prevent diabetes in NOD mice, but can we find a way that is relevant to human disease? I should say that for some of those agents, such as anti-CD3, anti-CD8, or some of the immunosuppressant molecules, one might find comfort or confidence in terms of disease prevention in terms of application of mice to humans. However, upon closer analysis, one finds that some unique ways have been able to show the ability to prevent disease in these animal models. Among those are treatments of phlebotomy, saline injections, or even manipulations of emotionality and cage shelf level (53).

In contrast to the ease at which type 1 diabetes prevention can occur in NOD mice, type 1 diabetes prevention as well as reversal in humans appears extremely difficult. Attempts to intervene in recent-onset diabetic patients with a variety of reagents, ranging from immunosuppressant compounds such as Cyclosporan and Imuran, to agents that modulate and stimulate the immune response (including *Bacillus* Calmette-Guerin), to agents that may even have an environmental link (such as dietary changes or vaccines), have all failed to show lasting effect in terms of spontaneous disease remission (54). This is not to say that the issue is entirely closed; a number of agents are being attempted for use in new-onset type 1 diabetic patients, and with time, we should know whether the reversal of disease is possible. Indeed, recent studies by Herold and Bluestone utilizing anti-CD3, for example, have been encouraging in their preliminary form (55). In addition to non–antigen-specific trials, a number of antigen-specific trials have been attempted or are ongoing. Here we are in a situation of reviewing the results of small pilot trials showing efficacy or awaiting further information once larger or more well-controlled trials have been completed. The NIH has given guidance and transitioned itself from the DPT-1 to NIH Trial Net. Trial Net will enhance the

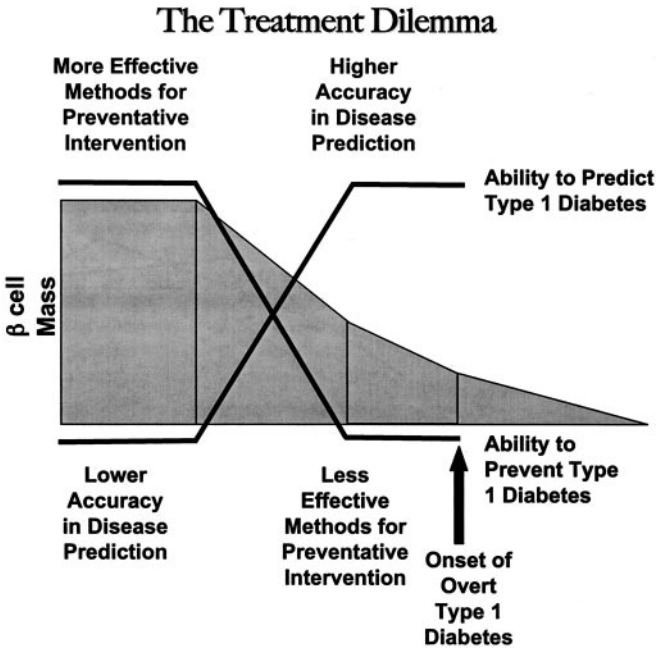

FIG. 6. The treatment dilemma for type 1 diabetes. A major ethical impediment to type 1 diabetes prevention remains finding the balance between risk and reward—treating those who are at risk for disease with an agent appropriate for their actual potential to develop type 1 diabetes. While an argument could be made for treatment of all those at increased risk for disease, barriers (both financial and ethical) exist to treat those who would never in actuality develop type 1 diabetes. Furthermore, experiences to date suggest that late interventions, at the point of symptomatic disease onset, may be less efficacious than early interventions. However, such a notion is extrapolated from the use of a limited number of agents in rodent models of type 1 diabetes, and it is hypothetical whether this, in fact, exists in humans.

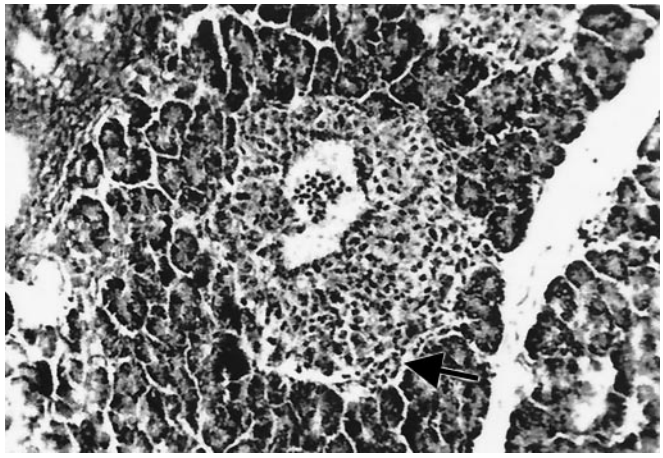

FIG. 7. Islet of Langerhans in human type 1 diabetes. Adapted from 1, shown is an additional pancreatic section demonstrating an islet of Langerhans from the patient described in Fig. 1. The authors portend that this represents a newly formed islet, developed around a dilated duct. A slight lymphocytic infiltration can be observed at the edge of the islet (arrow). Gomori's chromium hematoxylin-phlosine. Magnification ×150.

collective efforts of a series of individual laboratories, each chartered with the potential to bring new diabetes therapies aimed at prevention that would be tested in a series of adequately powered trials with well-defined laboratories and clinical monitoring analysis.

Why is the prevention of diabetes in mice relatively easy, whereas in humans it has proven remarkably difficult? One reason may be that we have overemphasized the similarities of the NOD mouse and human type 1 diabetes at the sake of recognizing the differences (12). Not that the similarities are not impressive. Indeed, both mice and humans share polygenic predisposition to the disease, defects in immune regulation, and the ability to induce disease remission with bone marrow transplantation. This is, as I coined a title with Ed Leiter, "as good as it gets" in terms of an animal model for type 1 diabetes (56). However, some crucial differences exist. The insulitis lesion may be different in the two disorders, that being mouse versus man, with NOD mice having a pathogenically benign lesion at phases in their disease. Studies suggest contrasting roles for maternal autoantibodies between mice and humans. Most striking may be the differences in the incidence and sex bias with the two diseases. Interestingly, this phenomenon is not restricted to rodent studies of type 1 diabetes, but has been observed in a variety of disorders. One reason for this is that the immune systems of mice and humans differ dramatically (57). Hence, it is possible that if such mechanisms are key to type 1 diabetes development, ignoring them in one model system may lead to disappointments when moving to translational studies.

What do we do to solve this puzzle? With this information in hand, the question is obvious: what do we do? In this lecture, I began with a series of predictions and assumptions that were present in the early days of our understanding the role for autoimmunity in type 1 diabetes and acknowledged our collective research successes, and shortcomings, in the ensuing decades. This type of action follows George S. Patton's advice of, "Prepare for the unknown by studying how others in the past have coped with the unforeseeable and predictable." Hence, as we move to the close of this talk, I would like to lay out a series of suggestions for solving this puzzle and developing a means to turn our understanding of the pathogenesis of type 1 diabetes into a means for preventing the disorder. Many of these suggestions target the areas previously mentioned as key puzzle pieces, and given that they have previously been discussed, will not be reiterated in great detail here rather than to say we need to find the "it," better define the natural history of type 1 diabetes, use animal models wisely, and to make disease prediction/prevention practical and cost effective.

To this latter issue, I will note that Dr. Desmond Schatz formed with me a model we call the treatment dilemma (Fig. 6). If one were to take the aforementioned natural history model and overlay on this figure two facets: first, the ability to predict type 1 diabetes; and second, the ability to prevent type 1 diabetes, one would observe two lines that run in opposite directions. Taken collectively, these lines form a dilemma. By this we mean that if we were to gauge our ability to predict future cases of type 1 diabetes, one would find that in the early stages of the disease process, our accuracy in disease prediction is lower than that in the period in which one approaches the symptomatic onset of type 1 diabetes. In contrast, extrapolation from existing data would lead one to speculate that that the earlier we were to treat with agents for immune intervention, the higher the resulting degree of efficacy. Likewise, in this model, as one moves closer to disease

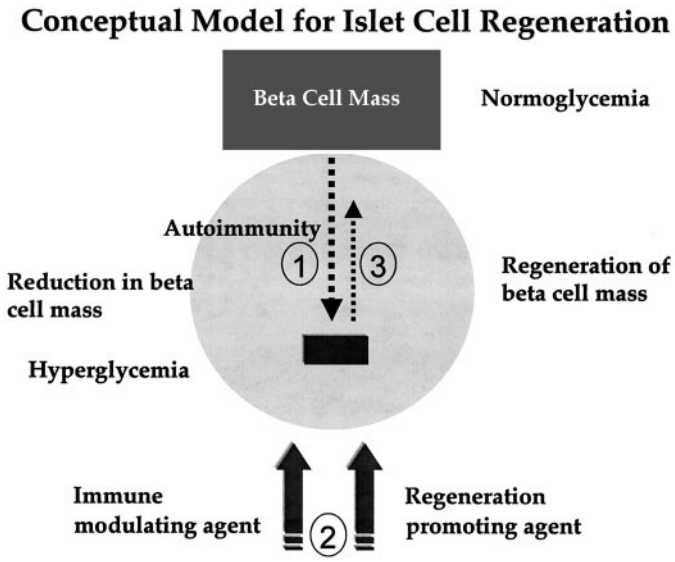

FIG. 8. Conceptual model for type 1 diabetes reversal. In this model, the mass of β-cells, due to the effects of autoimmunity, leads to a reduction in β-cell mass and hyperglycemia (step 1). The administration of an immunomodulatory agent alone, or in combination with an agent capable of inducing regeneration (step 2), could lead to the regeneration of β-cell mass (step 3) and the restoration of normoglycemia.

onset or even at the onset of type 1 diabetes, such therapies are less effective. Hence we are left with a dilemma. We can intervene early on in the onset of the disease and perhaps bring to the table more effective methods. However, such action would most likely involve the treatment in individuals who would not develop type 1 diabetes. However, a dilemna is formed if one takes the opposite approach, that being to only treat individuals who we regard with exceptionally high confidence. To solve this dilemma, future prevention efforts need to be realistic, truly realistic, in terms of their potential application to human disease.

While the best pieces to solve a puzzle may be the ones that go at the corners, there are other key pieces whose small bits of picture often lead to a definition of the big picture. These "practical" pieces are not only required to solve the puzzle, but given a shape and orientation that may not be so obvious, their importance may be unappreciated. For those pieces, I would portend that we need to once again look back at history to find the answer to this problem that has, to use slang, "dogged us" for 3 decades.

First, we may have overlooked or, at the very least, underappreciated a very important observation in those early descriptions of the pancreatic insulitis lesion. Indeed, 40 years ago it was noted that in the recent-onset type 1 diabetic pancreas, islet cells appeared to bud off pancreatic ducts (Fig. 7). Furthermore, insulin-positive tissue was even noted in the long-standing type 1 diabetic pancreas. We may have, for decades, underemphasized the potential to impart or enhance the endogenous regenerative capacity of the pancreatic β-cells. Future efforts must give emphasis to regeneration research.

Building from that model and adding the aforementioned observations that the onset of hyperglycemia may indeed be associated with more islet cell mass than previously thought, it would appear wise to target a model for islet cell regeneration in the new- or recent-onset type 1 diabetic patient (Fig. 8). As a starting place for impacting type 1 diabetes, this notion offers many practical advantages over disease prevention (i.e., we know who the subjects are and can avoid the practical barriers to screening, questions of risk/versus reward change, etc.). As we move into the area of disease reversal, it will also be interesting to see if such activities will be possible with immune-modulatory agents alone or in combination with any of an expanding series of agents (e.g., GLP-1 [glucagon-like peptide 1], exendin-4, etc.) that might be capable of imparting β-cell regeneration.

Finally, for a number of years, the notion of "suppressor T-cells" was a term and notion that fell out of favor in the immunology research community. With the observations by Sakaguchi et al. (58) that a population of T-cells marked by the coexpression of the CD4 and CD25 molecules were capable of imparting immune regulation, a breakout in research activities in this area has recently occurred. In the case of type 1 diabetes, we and others (38) have observed that type 1 diabetic patients appear to have inherent defects in this regulatory T-cell population. Yet, at the same time, additional studies have supported the notion that this population of T-cells may represent a key cell type to target for therapeutic studies aimed at disease prevention or reversal. Hence, efforts should be directed at the identification of cell therapies or agents capable of increasing the regulatory function of these cells for the purpose of preventing or reversing type 1 diabetes.

Additional avenues for hope. There are a number of other aspects of type 1 diabetes research that fall outside the bounds of this lecture that deserve at least one mention of recognition, for they too form the basis for a great deal of hope for the future. Hope is important; as Dale Carnegie said, "Most of the important things in the world have been accompanied by people who have kept on trying when there seemed no hope at all." Indeed, advances in agents allowing for tolerance induction may dramatically improve the steps taken forward in the Edmonton protocol for islet transplantation. Research in alternate sources of insulin-producing cells, from embryonic to adult stems cells, has exploded from a field that did not even exist a few years ago. Despite unfortunate setbacks that can come with the growth of any new field, I believe gene therapy will have its day, leading to improvements in a wide array of applications ranging from islet cell transplantation and noninvasive imaging to disease-associated complications. Thanks in large part to lobbying by organizations like the ADA, Congress has provided special appropriations for research in type 1 diabetes. As a result, the NIH and CDC (Centers for Disease Control and Prevention) have been able to coordinate and focus a number of programs for efforts aimed at identifying, in a collaborative and cooperative fashion, answers to the aforementioned puzzle pieces. Finally, just as technology makes our computers near obsolete within a short period of time, technological advances in methods of insulin delivery and glucose monitoring that seemed like pipe dreams just a few years ago are now entering the marketplace. It is time to be optimistic on a number of fronts, at least for those who will likely have rapid access to such treatments. Indeed, we should not forget that much of what I have

spoken about today is a mere fable, an unrealistic goal for many in the world with type 1 diabetes—patients who will, given the fate of the geographic location for which they happened to be born, will die for lack of insulin to treat their disease (59). For them, and I happen to be proud to say I have met, prayed, and cried with "them," the hope they hold would be for free access to the life-saving agent brought to us over 80 years ago for which we complain and try to break ourselves free from. For them, and us, I trust we find hope for a "cure" come to fruition.

Acknowledgments. In closing this Lilly Award presentation, I would like to end the discussion where it began. That being to provide a word of thanks to the multiple individuals who in addition to supporting me for this award, actually deserve recognition as sharing it with me. The contribution of these individuals to the studies I have just reviewed, as well as to finding a means to prevent and cure type 1 diabetes, have been incredibly large. I would like to recognize my family—Carol, Heather, Melanie, and Philip—individuals who have tirelessly sacrificed their own desires and needs for a husband and father for this cause. I also am proud to recognize my institutional colleagues and many other collaborators that have contributed to this cause over the years. Finally, a word to those who are stakeholders with a personal and vested interest in finding a cure for diabetes. Interacting with you and your family members has been a constant source of encouragement to me and remains the best part of being a diabetes researcher. To them, please know that we are trying, truly trying, and hopefully soon we will see an Outstanding Scientific Award lecture that describes how we, working together, uncovered a means to prevent and cure type 1 diabetes. Thank you.

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