



# Current Concepts on the Pathogenesis of Type 1 Diabetes—Considerations for Attempts to Prevent and Reverse the Disease

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## HISTORICAL MODEL OF TYPE 1 DIABETES PATHOGENESIS

It may be considered unusual to consider a period of three decades “historical.” Yet, the evolution for our understanding of the natural history and pathogenesis of type 1 diabetes has been greatly advanced by a vast number of studies aimed at validating a model (1), proposed by the late Dr. George Eisenbarth in 1986 (2). As a result of this work, the majority of current conventional wisdom portrays type 1 diabetes as a T cell–mediated autoimmune disease involving the specific destruction of insulin-producing pancreatic  $\beta$ -cells.

In this model, persons destined to develop type 1 diabetes are assumed to begin life with a full cadre of  $\beta$ -cells. However, a “triggering” insult, likely environmental, initiates a process involving the recruitment of antigen-presenting cells. Antigen-presenting cells sequester self-antigens released by injured  $\beta$ -cells, followed by their transport to pancreatic lymph nodes where they are subsequently presented to autoreactive T cells. These T cells, rogue constituents brought to life due to genetically driven failures of thymic deletion (i.e., central tolerance) combined with defects in mechanisms designed to induce peripheral immune tolerance, come into play (3). This toxic duo, imparting lack-of-tolerance formation, again in the context of genetic susceptibility, allows for migration of self-reactive T cells to islets, mediating  $\beta$ -cell killing and promoting further inflammation (4). When 85–90% of pancreatic  $\beta$ -cells meet their demise, symptoms of the disease occur. In the final stage of the model, the autoimmune process ends with the complete elimination of  $\beta$ -cells.

While this concept still forms the prevailing intellectual dogma for the majority of individuals associated with diabetes care and research today, a series of recent observations has challenged multiple aspects of this long-standing model (5). Many of these evolving concepts will be presented in this Perspective, with a discussion of how our understanding of models of type 1 diabetes pathogenesis has and will likely continue to evolve as it relates to attempts seeking to prevent and/or reverse the disorder.

## HOW HISTORICAL MODELS GUIDED PREVENTION AND REVERSAL STUDIES AND, POTENTIALLY, THEIR FAILURES

The timing for introduction of the Eisenbarth model appeared therapeutically “fortuitous” in its day. Contemporaneous with positing autoimmunity as the formative cause of type 1 diabetes in the 1980s were therapeutic interventions developed for organ transplantation. This research brought forward a series of immunosuppressive agents thought clinically promising for multiple immune based–disorders, including type 1 diabetes. The earliest of the immunosuppressive-based studies in type 1 diabetes, using agents such as cyclosporine or azathioprine, provided evidence that preservation of endogenous insulin secretion was possible, even if only for a relatively short period of time, in settings of recent-onset disease. Such news brought hope that a means to prevent or cure type 1 diabetes was on the horizon. While adverse effects of these agents brought a close to their use in type 1 diabetes,

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they set the stage for a multidecade effort to utilize a variety of biologics to target autoimmunity for the purpose of stemming the tide of  $\beta$ -cell destruction.

Clinical trials seeking to meet the goal of type 1 diabetes prevention (Table 1) (recently reviewed in refs. 6–9) in the case of autoantibody-positive subjects with type 1 diabetes or C-peptide preservation in recent-onset subjects (Table 2) (recently reviewed in ref. 10) have been quite variable in terms of their “success.” While some trials have demonstrated the ability to either delay progression to type 1 diabetes or preserve C-peptide production in individuals with recent-onset type 1 diabetes, the vast majority of such efforts has either failed to meet the predetermined end points, or even when demonstrating early success (i.e., meeting goals of C-peptide production at 12 months for subjects with type 1 diabetes), loss in C-peptide production eventually occurs for most. Mechanistic studies affiliated with these efforts have, to a large extent, failed to identify specific mechanisms associated with therapeutic failure or success. The failures in achieving therapeutic success in humans stand in stark contrast to the results of studies in the NOD mouse,

where methods capable of preventing type 1 diabetes and/or reversing overt hyperglycemia abound (11–13). We would suggest that many of the failures of human studies have been the by-product of having a poor understanding of the complexity of the disorder’s pathogenesis—too many factors have historically been underappreciated, misunderstood, or unknown in considerations of the pathogenesis of type 1 diabetes (Table 3).

### EMERGING VIEWS ON THE ROLE FOR IMMUNE RESPONSES IN TYPE 1 DIABETES

Perhaps no segment of the historical model for type 1 diabetes pathogenesis has been as rigorously investigated as that of the immune response of persons with or at various levels of risk for the disease. While such studies have yielded success stories with practical outcomes (e.g., autoantibody staging for disease risk, biomarker development, identification of subjects for disease prevention trials), they all suffer from a variety of limitations. By their nature, nearly all studies of human immune responses involve analysis of peripheral blood rather than at the site of  $\beta$ -cell destruction. In addition, while the potential importance of the so-called effector and regulatory

components in type 1 diabetes pathogenesis have been extensively studied, only recently have serious considerations been given to the effects of aging, diet, immune cell metabolism, microbial pathogens, microbiomes, and epigenetic changes on the immune response affording this disease (Table 3) (14–18). These factors, individually and in combination, clearly influence immune responses in general and, thus, must be associated by default with the pathogenesis of type 1 diabetes.

As a number of type 1 diabetes reviews have recently been published highlighting features of the immune response in peripheral blood (4,19,20), this Perspective will focus on studies of human pancreas and other tissues obtained from organ donors with or at risk for type 1 diabetes that have largely, but not exclusively, been made possible through the efforts of the Belgian Beta Cell Bank and the JDRF Network for Pancreatic Organ Donors with Diabetes (nPOD) program (21,22).

### Immunological Characteristics of the Pancreas in Type 1 Diabetes—Evidence for Disease Subtypes

Recent studies of human pancreata have added support to the growing

**Table 1—Prevention trials in type 1 diabetes**

Study name	Intervention	1°Outcome	End point achieved	Reference or ClinicalTrials.gov identifier
<b>Primary prevention studies</b>				
NIP	Dietary docosahexaenoic acid	Pilot study	— **	60
Finnish TRIGR pilot	Hydrolyzed casein formula	Autoantibodies	Yes	61
BABYDIET	Delayed dietary gluten exposure	Autoantibodies	No	62
TRIGR	Hydrolyzed casein formula	Autoantibodies	No	63
FINDIA	Whey-based, insulin-free bovine milk formula	Autoantibodies	Yes	64
<b>Secondary prevention studies</b>				
DENIS	Nicotinamide	Diagnosis of type 1 diabetes	No	65
DPT-1 Parenteral Insulin	Parenteral insulin	Diagnosis of type 1 diabetes	No	66
INIT I	Intranasal insulin	Safety	Yes#	67
ENDIT	Nicotinamide	Diagnosis of type 1 diabetes	No	68
DPT-1 Oral Insulin	Oral insulin	Diagnosis of type 1 diabetes	No	69
DIPP sibling cohort	Intranasal insulin	Diagnosis of type 1 diabetes	No	70
DIPP birth cohort	Intranasal insulin	Diagnosis of type 1 diabetes	No	70
Belgian Parenteral Insulin	Parenteral insulin	Diagnosis of type 1 diabetes	No	71
TrialNet Oral Insulin	Oral insulin	Diagnosis of type 1 diabetes	*	NCT00419562
INIT II	Intranasal insulin	Diagnosis of type 1 diabetes	*	NCT00336674
DIAPREV-IT	GAD-alum (Diamyd)	Diagnosis of type 1 diabetes	*	NCT01122446
TrialNet Teplizumab	Teplizumab	Diagnosis of type 1 diabetes	*	NCT01030861
TrialNet Abatacept	CTLA4lg (abatacept)	Diagnosis of type 1 diabetes	*	NCT01773707

Adapted with permission from Skyler (9). DENIS, Deutsche Nicotinamide Intervention Study; DIAPREV-IT, Diabetes Prevention—Immune Tolerance; DIPP, Type 1 Diabetes Prediction and Prevention Project; DPT-1, Diabetes Prevention Trial—Type 1; ENDIT, European Nicotinamide Diabetes Intervention Trial; FINDIA, Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes; INIT, Intranasal Insulin Trial; NIP, Nutritional Intervention to Prevent Diabetes; TRIGR, Trial to Reduce IDDM in the Genetically at Risk. \*Data not yet available. \*\*Pilot study. #No adverse events.

**Table 2—Reversal trials in type 1 diabetes**

Study name	Treatment(s)	C-peptide at 1 year (nmol/L)	Preservation of C-peptide♦	Reference
Cyclosporin Treatment in Children with Recent-Onset Type 1 Diabetes <sup>o</sup>	Cyclosporin A	0.3	Yes*	72
Continuous Insulin Infusion Throughout the First Two Weeks Following Type 1 Diabetes Onset <sup>o</sup>	Intensive insulin therapy	0.5	Yes	73
IMDIAB IV	Vitamin E	0.2	Yes	74
IMDIAB VI	Nicotinamide	0.2	Yes	75
Diabète Insuline Orale	Oral insulin	0.1	No	76
DIA-AID2	DiaPep277	0.2	Yes	77
AbATE	hOKT3gamma1(Ala-Ala)	0.2	Yes	78
Diazoxide Treatment in Children with New-Onset Type 1 Diabetes <sup>o</sup>	Diazoxide	0.2	Yes	79
The Use of Polyclonal Anti-T-Lymphocyte Globulin to Prevent Progression of Autoimmune $\beta$ -Cell Destruction in Recent Type 1 Diabetes	ATG	0.2	Yes	80
IMDIAB IX	Nicotinamide + vitamin E	0.2	Yes**	81
IMDIAB (retrospective analysis)	Nicotinamide + intensive insulin therapy	0.1	Yes**	82
TTEDD	ChAglyCD3 (otelixizumab)	0.5	Yes	83
Phase II Trial of hOKT3gamma1(Ala-Ala) Teplizumab for Treatment of Patients With Recent Onset Type 1 Diabetes	hOKT3gamma1(Ala-Ala)	0.2	Yes	84
IMDIAB XI	Calcitriol + nicotinamide	0.1	No	85
DIA-AID2	DiaPep277	0.2	Yes§	86
Autologous Hematopoietic Stem Cell Transplantation in Type 1 Diabetes Mellitus	AHSCT	0.3	Yes	87
Phase II Trial of DiaPep277 in Children with New-Onset Type 1 Diabetes <sup>o</sup>	DiaPep277	0.2	No	88
DIA-AID2	DiaPep277	0.4	Yes (trend)#	89
A Phase II, Randomised, Double-Blind, Placebo-Controlled, Multi-Centre Study to Investigate the Impact of Diamyd on the Progression of Diabetes in Patients Newly Diagnosed With Type 1 Diabetes Mellitus	GAD-alum	0.1	No	90
Phase II Multiple Dose Treatment of Type 1 Diabetes Mellitus With hOKT3gamma1(Ala-Ala)	Teplizumab	0.8	Yes (trend)	91
A Randomized, Double-Blind, Placebo-Controlled, Multicenter, Parallel, Dose-Ranging Study to Evaluate the Efficacy, Safety, Tolerability, and Pharmacodynamics of NBI-6024 In Adult and Adolescent Patients With New Onset Type 1 Diabetes Mellitus	NBI-6024	0.1	No	92
Autologous Hematopoietic Stem Cell Transplantation in Type 1 Diabetes Mellitus	AHSCT	AUC = 30	Yes	53
Effects of Rituximab on the Progression of Type 1 Diabetes in New Onset Subjects	Rituximab	0.1	Yes¥	93
ENBREL (Etanercept) Administration to Patients Newly Diagnosed With Type 1 Diabetes Mellitus: Feasibility-Safety Study	Etanercept	0.4	Yes	94
Extension of Phase II Therapeutic Trial With a Humanized Non-Mitogenic CD3 (ChAgly CD3) Monoclonal Antibody in Recently Diagnosed Type 1 Diabetic Patients	ChAgly CD3	AUC = 0.9	Yes#	95
Efficacy of 6 Months Treatment With Diazoxide at Bedtime in Preventing $\beta$ -Cell Demise in Newly Diagnosed Type 1 Diabetes	Diazoxide	0.1	No	96
Immunointervention With 1,25-dihydroxy-vitamin D3 in New-Onset Type 1 Diabetes	1,25(OH) <sub>2</sub> D <sub>3</sub>	0.1	No	97

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Table 2—Continued

Study name	Treatment(s)	C-peptide at 1 year (nmol/L)	Preservation of C-peptide <sup>◆</sup>	Reference
Protégé study	Teplizumab	0.5	Yes <sup>#</sup>	98
TrialNet GAD	GAD-alum	0.3	No	99
TrialNet Abatacept	Abatacept	0.3	No	100
DIATOR	Atorvastatin	0.2	No	101
Efficacy of ATG + Autologous CD34 <sup>+</sup> Stem Cells + GCSF in New-Onset Type 1 Diabetes <sup>°</sup>	Mobilized hematopoietic CD34 <sup>+</sup> stem cells	0.4	Yes	102
A Phase III, 3-Arm, Randomized, Double-Blind, Placebo-Controlled, Multicenter Study to Investigate the Impact of Diamyd on the Progression of Diabetes in Patients Newly Diagnosed With Type 1 Diabetes Mellitus (EU)	GAD-alum	0.3	No	103
DIATOR	Atorvastatin	0.2	Yes <sup>†</sup>	104
Prospective Study of Autologous Hematopoietic Stem Cell Transplantation to Treat New Onset Type 1 Diabetes	AHSCT	0.6	Yes	105
Safety and Efficacy Study of Autologous Nonmyeloablative Hematopoietic Stem Cell Transplantation for Early Onset Type 1 Diabetes—A Phase II Study	AHSCT		Yes	106
A Phase I Trial of Proleukin and Rapamune in Recent-Onset Type 1 Diabetes Mellitus (ITN018A1)	Rapamycin/interleukin (IL)-2	AUC = 6.3	No	107
Canakinumab Study in Individuals With Newly Diagnosed Type 1 Diabetes (anti-IL-1)/ Anti-Interleukin-1 in Diabetes Action	Canakinumab/Anakinra	0.1	No	108
Reversing Type 1 Diabetes After it is Established: A Pilot Safety and Feasibility Study of Anti-Thymocyte Globulin (Thymoglobulin) and Pegylated GCSF (Neulasta) in Established Type 1 Diabetes	ATG + GCSF	0.74	Yes	54

Adapted with permission from Ben Nasr et al. (10). AbATE, Autoimmunity-Blocking Antibody for Tolerance in Recently Diagnosed Type 1 Diabetes; AHSCT, autologous hematopoietic stem cell transplantation; ATG, anti-T-lymphocyte globulin; AUC, area under the curve; DIA-AID, Efficacy and Safety Study of DiaPep277 in Newly Diagnosed Type 1 Diabetes Adults; DIATOR, Diabetes Intervention With Atorvastatin; GCSF, granulocyte-colony stimulating factor; IMDIAB, Immunotherapy of DIABetes; TTED, TRX4 Monoclonal Antibody in Type 1 Diabetes (T1 DM). <sup>◆</sup>Defined as author-reported interpretation. <sup>°</sup>Official study name could not be determined. <sup>\*</sup>Effect lost on withdrawal of treatment. <sup>\*\*</sup>No synergistic benefit with combination. <sup>§</sup>Differential outcomes depending on dose. <sup>#</sup>Differential effects depending on age-group. <sup>‡</sup>Reported partial preservation of  $\beta$ -cell function. <sup>†</sup>Differential effects depending on baseline C-reactive protein concentrations.

concept that subtypes of type 1 diabetes truly exist. For example, when histological studies of type 1 diabetes pancreata are combined, patients with disease onset at age 0–14 years and within 1 year of diagnosis show more inflamed islets (68%) and fewer islets with residual

$\beta$ -cells (39%) than do patients with onset at 15–39 years of age (23). This suggests a more vigorous autoimmune response occurs when disease develops in young children.

Additional evidence in support this concept was the recent finding that

younger age of onset is associated with higher levels of CD20<sup>+</sup> B cells, CD45<sup>+</sup> cells, and CD8<sup>+</sup> T cells in insulinitis lesions, with fewer insulin-positive islets (15,24). Conversely, infiltrates with fewer CD20<sup>+</sup> cells were observed in patients with type 1 diabetes who were

Table 3—Features influencing the pathogenesis and natural history of type 1 diabetes likely underappreciated in therapeutic trials seeking to prevent and or reverse the disease

Immune	Pancreas/ $\beta$ -Cells	Environment/Genetics
Innate immunity	Small pancreas	Microbiome (gut, oral)
Influence of age on immune response	Vascular abnormalities	Diet
Immune cell metabolism	Pancreatitis (role)	Antibiotic use
Acute versus chronic $\beta$ -cell destruction	Exocrine infiltration	Exercise
Limited/focal nature of insulinitis	$\beta$ -Cell replication as a function of age	Epigenetic modifications Relationship with gut (including celiac disease)

older at onset and were associated with lower levels of CD45<sup>+</sup> cells and CD8<sup>+</sup> T cells, as well as more insulin-positive islets. These same studies also noted that islet CD8<sup>+</sup> T cells expressed T-cell receptors that bound MHC class I tetramers loaded with the  $\beta$ -cell autoantigen IGRP and other target peptides in patients with recent-onset type 1 diabetes (24). These studies are also in agreement with recent findings that the T cells invading pancreatic islets are, in fact, directed at  $\beta$ -cell antigens (25).

It is also noteworthy that pancreata from patients in each age-group greater than 1 year from diabetes onset have similarly low levels of insulinitis (3–4%) and equivalent maintenance of insulin-positive islets (13%). Therefore, it appears that the process of insulinitis formation in the two age-groups may have equalized and lessened over time, avoiding complete  $\beta$ -cell annihilation. As discussed below, recent studies indicate that some  $\beta$ -cells survive in type 1 diabetes for many decades (24,26). Thus, key questions become: What type of inflammation is present in these disease subtypes at various stages at what stages and can these be pushed toward the resolution phase that may be part and parcel of the inflammatory process?

#### Inflammation Is Also Present in Pancreatic Exocrine Tissue

Recent studies emanating from nPOD demonstrate chronic inflammation, including enhanced CD8<sup>+</sup> T-cell infiltration (and, to a lesser degree, CD4<sup>+</sup> and CD11c<sup>+</sup> cells) in the exocrine pancreas in subjects with type 1 diabetes (27). Other studies report a similar propensity for neutrophil invasion of the pancreas (with decreased peripheral neutrophil counts) in type 1 diabetes (28). The propensity of this organ for inflammation/pancreatitis induced by multiple factors (e.g., hypertriglyceridemia, virus infection, drugs) could be a result of a susceptibility gene that affects tissue-based inflammation or other facets that have, in the past, been considered “leakiness” (29,30). Importantly, the inflammatory process in the pancreas appears to be subclinical, as most new-onset patients do not present with symptoms of pancreatic inflammation.

#### Clarifying the Immune: Viral Connections in Type 1 Diabetes

While numerous viruses have been posed as potential contributors to the

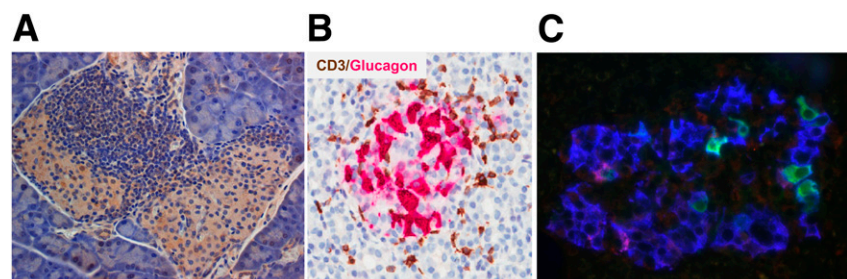
pathogenesis of type 1 diabetes, the vast majority of investigation continues to focus on coxsackievirus (CVB). Indeed, recent efforts have provided evidence for the presence of viral capsid protein expression, VP1, as well as viral RNA and type 1 interferon (IFN) in islets (27,31).

Recently, we and others have noted expression of multiple type 1 IFN signature proteins, including MDA5, in association with CVB capsid protein, VP1, in type 1 diabetes pancreata (32,33). The islets in new-onset organ donors (Fig. 1B) and in autoantibody-positive donors (data not shown) demonstrate infiltrate; however, it is substantially less than that seen in the NOD mouse (Fig. 1A). In addition, immune cells tend to invade the NOD islet but manifest largely as perinsulinitis in humans (Fig. 1A and B). In both NOD (data not shown) and the islets from humans with new-onset disease, there is evidence for type 1 IFN activity as MDA5 and other IFN-signature proteins are expressed (Fig. 1C). However, in pancreata from patients with diabetes, for several years there are many insulin-positive cells, MDA5-positive islet cells, and VP1 capsid protein all expressed in the absence of CD45<sup>+</sup> cell infiltration (Fig. 1C and M.C.-S., unpublished data). These data suggest a chronic, persistent, low-level viral infection may perpetuate a form of islet inflammation long-term that is tissue based. As to potential underlying mechanisms for such observations, CVB RNA may undergo 5' terminal deletions in

vivo, leading to a replication-deficient virus, affording a potential explanation for viral persistence and lack of immune activation resulting in viral clearance (34). Alternatively, genetic contributions to acute viral responses regulated by type 1 diabetes susceptibility genes (e.g., *IFIH1*, *Tyk2*, or *PTPN22*) or increased numbers of plasmacytoid dendritic cells producing high levels of type 1 IFN may modulate downstream antiviral responses (32,35). Persistent viral infections were recently shown to propagate chronic type 1 IFN responses that downgrade inflammation and immune responses, thereby contributing to continued viral infection (36–38). If CVB or other viral infections cause chronic type 1 IFN-based inflammation, immune modulation of this response or the use of antiviral therapy may provide a means to clear the virus or reduce the inflammation that affects  $\beta$ -cell function and/or survival.

#### EMERGING VIEWS ON THE ROLE FOR $\beta$ -CELLS IN TYPE 1 DIABETES

Over the past decade, knowledge regarding human pancreatic islets and  $\beta$ -cells has increased dramatically. Amplified by studies on human pancreatic islets from the National Institutes of Health (NIH) Integrated Islet Distribution Program (IIDP) and nPOD, a greater understanding of human islet gene expression, function, proliferation, and regeneration has emerged. A consensus has materialized that human pancreatic islets have fundamental differences



**Figure 1**—Emerging features regarding pancreatic islets in type 1 diabetes. Recent descriptions of insulinitis (23,25,51,55) have placed an emphasis on the quantitative differences in this lesion when comparing human pancreatic samples to those observed in the NOD mouse model of disease. For example, the intensity and pattern of lymphocytic infiltration in NOD mice at or immediately prior to disease onset (A; 14-week-old new-onset case) is quite pronounced relative to that of human type 1 diabetes (B; 13-year-old with type 1 diabetes <1 year, nPOD 6228). C: Consistent with a notion ascribing a role for viral infections with type 1 diabetes, an nPOD organ donor from a patient with disease onset at 10.2 years of age and a 4-year duration was examined. An islet from this donor expressed abundant insulin (blue), CVB capsid protein VP1 (green), and MDA5 (red) in islet cells.  $\beta$ -Cells expressing both MDA5 and insulin are purple. This islet was also negative for CD45 staining, demonstrating a lack of insulinitis (representative image = 40 $\times$ ).

with the more widely studied rodent islet, including dissimilarities in islet cell composition, basal insulin secretion, susceptibility to toxins such as streptozotocin, amyloid formation, and cell proliferation (39–42).

In response to the increased metabolic demands of insulin resistance and obesity, rodent  $\beta$ -cells increase insulin biosynthesis and cellular proliferation, leading to a marked increase in  $\beta$ -cell mass. In contrast, pancreatic samples from obese humans show only a minimal or modest expansion in  $\beta$ - or islet-cell mass (43). These differences between rodent and human islets do not invalidate rodent models of islet biology but require increased attention and integration of findings in rodents to human islets and the human pancreas.

### $\beta$ -Cell Mass Is Not Equal in All Individuals

Historical models of type 1 diabetes have assumed a normal  $\beta$ -cell mass at birth that declines once the autoimmune attack occurs. However, recent studies of cadaveric pancreata have shown that  $\beta$ -cell mass in normal humans without diabetes varies three- to fivefold, independent of adult age or BMI, with  $\beta$ -cell mass likely mostly determined in the first two decades of life (43–45). This has important implications when one considers the

starting point for declining  $\beta$ -cell mass during autoimmune  $\beta$ -cell destruction. Thus, an individual's timeline to diabetes onset could be determined not by the severity of the autoimmune attack but the starting point for  $\beta$ -cell mass (Fig. 2). The reasons for this variation in  $\beta$ -cell mass are unknown but could include the in utero environment, events during the first decade of life, and yet unknown genetic or environmental determinants. Further emphasizing the need to understand the timeline and determinants of human  $\beta$ -cell mass is the recent observation of a smaller pancreatic mass in individuals with new-onset type 1 diabetes or with islet-cell autoantibodies (46,47). This observation raises the possibility that determinants of both pancreatic mass and  $\beta$ -cell mass might be impacted, as endocrine islet cells and exocrine cells share a common embryologic heritage.

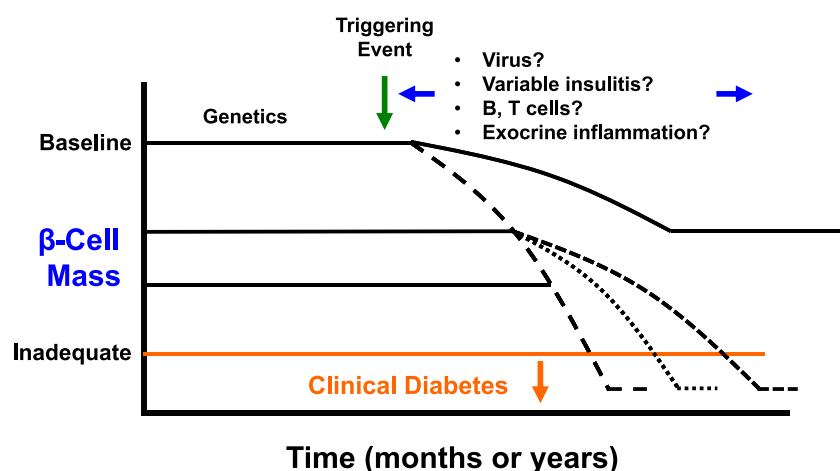
### Are All $\beta$ -Cells Equally Susceptible to Destruction in Type 1 Diabetes?

It has also been assumed that all human  $\beta$ -cells are equally susceptible to autoimmune attack and that differences in the timeline of type 1 diabetes pathogenesis relate to immune differences. In reality, variations in  $\beta$ -cell susceptibility to cytokines or immune cell attack could be an important determinant of when an individual develops clinical diabetes

(Fig. 2). While certain immunomodulatory approaches appear to improve C-peptide production (6), improved  $\beta$ -cell function is not synonymous with prevention of  $\beta$ -cell loss or recovery of  $\beta$ -cell mass. An ongoing debate surrounding type 2 diabetes is whether loss of  $\beta$ -cell function or reduction in mass is the reason for inadequate insulin secretion, but most agree that both pathogenic processes are important. In addition, metabolic derangements clearly impact key islet-enriched transcription factors or may promote loss of  $\beta$ -cell identity (48,49). Therefore, potential parallels with  $\beta$ -cell dysfunction and/or loss in type 1 diabetes seem clear. A challenge is that there are no markers (other than insulin secretion) or noninvasive imaging modalities that reflect  $\beta$ -cell mass in humans. As insulin secretion (basal or stimulated as part of the intravenous glucose tolerance test, oral glucose tolerance test, or mixed-meal tolerance test) can be affected by chronic elevations in the blood glucose and secretory capacity has not been shown to truly correlate closely with  $\beta$ -cell mass over time, improvements in how to assess  $\beta$ -cell mass in humans are needed.

### Subjects With Long-standing Type 1 Diabetes, in Fact, Have $\beta$ -Cells

One of the longer-standing dogmas in type 1 diabetes is that eventually all  $\beta$ -cells are lost in long-standing disease, but the emerging reality is quite different. Many individuals with type 1 diabetes produce small amounts of C-peptide, and studies of the pancreata from individuals with type 1 diabetes show the presence of insulin-positive cells, sometimes within glucagon-rich islets or as single insulin-positive cells scattered throughout the pancreatic exocrine tissue (24,26,50). Interestingly, C-peptide levels were higher in patients >18 years of age at onset and with shorter duration of diabetes (26). These findings raise the question of why some  $\beta$ -cells escape the autoimmune attack or are somehow resistant to it. Are the surviving  $\beta$ -cells somehow "different"? Alternatively, new  $\beta$ -cells may be constantly being regenerated and subsequently destroyed by the ongoing autoimmune process. Now that the transcriptional profile and molecular signatures of normal human  $\beta$ -cells are being defined, it should be possible to determine whether these residual insulin-positive cells are "normal"  $\beta$ -cells.



**Figure 2**—Summary of recent changes in our understanding of the pathogenesis of type 1 diabetes. The y-axis shows  $\beta$ -cell mass in three individuals with a different baseline  $\beta$ -cell mass over time (x-axis). Likewise, the loss of  $\beta$ -cell mass may differ among individuals as shown by the dashed lines. Finally,  $\beta$ -cell mass is markedly inadequate, but does not become “zero” in many individuals with type 1 diabetes as shown by the horizontal dashed lines. While these lines are drawn as smooth, it is likely that the decline in  $\beta$ -cell mass or function is intermittent and possibly episodic. Recent discoveries also highlight the presence of viral particles in the type 1 pancreas, the variable insulinitis, the role of B and T lymphocytes, and the presence of inflammatory cells in the exocrine pancreas. These processes are shown as occurring after an unknown “triggering event,” but this is speculative.

### Inducing Human $\beta$ -Cells to Proliferate

Intense efforts to induce human  $\beta$ -cell proliferation are under way and our improved understanding of human  $\beta$ -cell biology is providing clues regarding signaling pathways and cell cycle determinants important for human  $\beta$ -cell proliferation (42–44). As recently summarized, we currently lack an approach to induce sustained human  $\beta$ -cell proliferation with an acceptable safety profile (42). A clear challenge is the need to induce only  $\beta$ -cell proliferation as many of the current approaches and growth factors being tested target pathways present in many cell types. The ability to specifically target  $\beta$ -cells in vivo with either a proliferative signal or a protective intervention is needed. Moreover, successful strategies in prevention of type 1 diabetes and/or in preservation of  $\beta$ -cell function may require interventions targeting immune pathways in combination with approaches that promote  $\beta$ -cell proliferation (Fig. 3).

### HOW KNOWLEDGE REGARDING THE PATHOGENESIS AND NATURAL HISTORY OF TYPE 1 DIABETES IS VITAL FOR EFFORTS TO PREVENT AND REVERSE THE DISEASE

#### When and How Fast Are $\beta$ -Cells Lost in Type 1 Diabetes and Is It a True Loss That Occurs or a Mere Loss of Their Function?

At odds with studies of NOD mice (Fig. 1A), it has been difficult to document insulinitis in many type 1 diabetes cases during the prediabetic phase (Fig. 1B), when individuals already have clear signs of autoimmunity (51). This observation implies that attacks on  $\beta$ -cells likely occur in a relapsing-remitting fashion (52). As currently available biomarkers fail to indicate precisely when periods of attack occur, such periods could be missed with short-term therapies. Therefore, future trials should consider longer-term treatment periods or utilization of agents whose effects would be lasting (e.g., tolerance inducing). However, agents used for long-term treatment must also avoid adverse side effects in order to gain widespread acceptance.

It has also recently become apparent that  $\beta$ -cell mass does not decrease in a linear fashion (T. Rodriguez-Calvo, K. Herold, M.A.A., and M.v.H., unpublished data). Indeed, substantial  $\beta$ -cell mass might still be

present until just prior to the time when oral glucose tolerance testing becomes abnormal. This latter observation is potentially encouraging in that more  $\beta$ -cells may be present than once thought prior to diagnosis. As a result, efforts to preserve  $\beta$ -cell mass and metabolic capacity in settings of secondary disease prevention might have more potential for success than previously assumed.

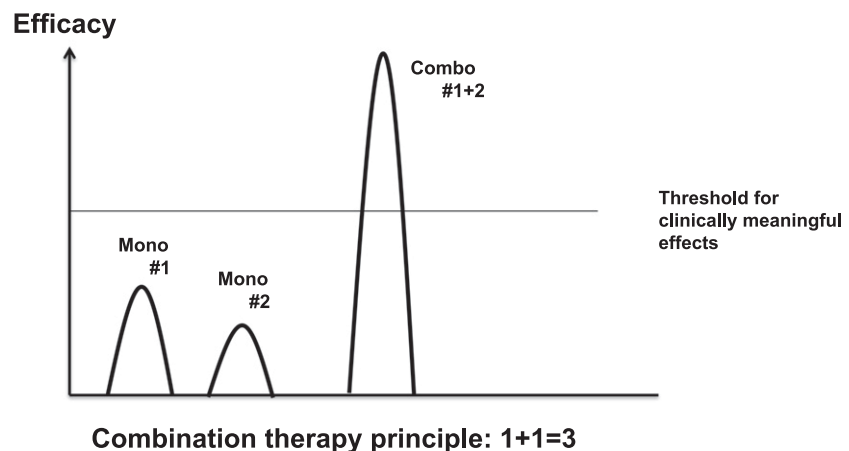
Questions also abound regarding the degree of  $\beta$ -cell function (or lack thereof) following diagnosis of the disease. It was previously thought that approximately 90% of  $\beta$ -cell mass and function are irrevocably lost by the time type 1 diabetes is diagnosed. However, we now know that strong immune suppression can result in a rather rapid recovery of  $\beta$ -cell function (53). Factors that contribute to reduced  $\beta$ -cell function at the time of diagnosis include inflammatory stress, excessive demand for insulin, and endoplasmic reticulum stress—deleterious processes that are at least in part reversible. Furthermore, as noted previously, many adults with type 1 diabetes of extended duration still retain a degree of C-peptide production (54). This realization points toward the possibility that maintenance of remaining  $\beta$ -cell function in adults, even many years postdiagnosis, may provide clinical benefit.

#### Nature of the Beast—Who Attacks and Destroys the $\beta$ -Cells and How Do We Need to Deal With It?

The most prominent cell found in human islets in the setting of type 1

diabetes is the CD8<sup>+</sup> cytotoxic T cell, which is also a likely candidate to aid in  $\beta$ -cell killing due to its ability to recognize targets via antigen in the context of MHC class I, which is elevated in many islets in those with the disease (55). Therapeutically, lymphocytes and memory lymphocytes of the adaptive immune response can be targeted by anti-T-cell drugs, such as anti-CD3, anti-CD2 (LFA3Ig), and certain costimulatory blockers (56,57). Indeed, partial success of such compounds in recently diagnosed type 1 diabetes, defined by preservation of  $\beta$ -cell function (i.e., C-peptide production) over several months to years, speaks toward an important role for such autoreactive lymphocytes in  $\beta$ -cell destruction, at least late during the pathogenesis of type 1 diabetes.

Are  $\beta$ -cell antigen-specific CD8<sup>+</sup> T cells the only factor? Certainly not, as it has become clear that general low-grade inflammation can be observed in the exocrine pancreas and inflammatory cytokines known to harm islets are also thought to be elevated during type 1 diabetes pathogenesis. Thus, anti-inflammatory therapies targeting cytokines may hold promise, and a recent trial blocking tumor necrosis factor has shown initial promise in preserving  $\beta$ -cells (58). These observations provide further support for the concept of combination therapies. Examples of such combinations would include an induction component using drugs targeting



**Figure 3**—A conceptual model for improving efforts to prevent and or cure type 1 diabetes. Previous clinical trials using monotherapies (i.e., single agents) have shown various degrees of success, albeit even in the best of situations partial, in terms of achieving desired therapeutic outcomes. Here, we diagram the potential, albeit a theoretical, model for the principle that combination therapies would provide a substantial improvement over singular monotherapies, with respect to preservation of  $\beta$ -cell mass. Mono, monotherapy.

inflammation and T-/B-cell memory, as well as a maintenance component that could involve antigens to induce tolerance to  $\beta$ -cells.

One additional important question involves how the  $\beta$ -cell appears on the radar screen of the immune system in the first place. Is autoimmunity the primary cause, or might it be that metabolic derailment exerts stress on  $\beta$ -cells and in this way makes them visible to the immune system? In reality, this might at least be a contributing factor to the pathogenesis of type 1 diabetes and type 2 diabetes, as metabolic markers can precede the diagnosis of the former by several years. Considering this, priority should be given to the addition of drugs to combination therapies that stabilize and maintain  $\beta$ -cells and  $\beta$ -cell function (Fig. 3).

## CONCLUSIONS AND FUTURE DIRECTIONS

Within a few years, those involved in the care of persons with type 1 diabetes as well as researchers seeking to make impactful discoveries for those living with the disease will celebrate the centennial anniversary of the discovery of therapeutic insulin. Thankfully, the era since that monumental event has seen a multitude of improvements in diabetes care (59). At the same time, significant research efforts have been directed at finding the underlying cause(s) of type 1 diabetes, in large part guided by the notion of developing a means to prevent as well as provide a true “cure” for the disease. While progress has clearly been made toward understanding the initiating and sustaining events in the pathogenesis of type 1 diabetes (Fig. 2), much more investigation and discovery are needed. We believe that future attempts to prevent and/or reverse type 1 diabetes are most likely to be successful if they incorporate the recent advances in our evolving understanding of pathogenesis of the disease.

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