PREVENTION OF TYPE 1 DIABETES

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SUMMARY

Type 1 diabetes is a progressive disease. There is a genetic predisposition to type 1 diabetes, particularly conferred by alleles present within the major histocompatibility complex (HLA region) on the short arm of chromosome six. It is thought that in susceptible individuals, an environmental trigger initiates an immune response. Immune infiltration into pancreatic islets results in beta cell damage, impairment of beta cell function, and potential destruction of beta cells. One would expect that if type 1 diabetes is an immunologically mediated disease, then immune intervention should alter the natural history of the disease and potentially abrogate the clinical syndrome.

Intervention trials have been conducted at a number of stages of the disease process. Primary prevention trials have been conducted in individuals with a genetic predisposition who have not yet developed immunologic markers ("Pre-Stage 1"). Secondary prevention trials have been conducted in individuals with two or more diabetes-related autoantibodies, either during Stage 1 of type 1 diabetes (normal metabolic function) or Stage 2 of type 1 diabetes (abnormal metabolic function). Intervention trials, also called tertiary prevention trials, have been conducted in individuals with Stage 3 of type 1 diabetes (clinical hyperglycemia), usually shortly after clinical onset of disease.

This chapter provides brief summaries of the randomized controlled clinical trials that have been conducted and also mentions some non-randomized pilot studies. Unfortunately, none of the primary or secondary prevention trials have clearly arrested the disease process. Some tertiary intervention trials have demonstrated improved beta cell function, at least for some period of time, after which beta cell function has generally declined in parallel to that in the respective control group. This could be a consequence of most studies focusing on only a single immunologic mechanism; whereas, what may be required are studies that deal with multiple immunologic mechanisms, including attempting to improve regulatory immunity, while also addressing beta cell function by including interventions that improve beta cell health.

INTRODUCTION

Type 1 diabetes is a slowly progressive disease, with a genetic predisposition, where a putative environmental trigger initiates an immune response that results in pancreatic islet beta cell damage, impairment of beta cell function, and destruction of beta cells (1,2,3). Although the initial characterization suggested a slow, linear progression of disease (Figure 37.1) (1), more recent thought is that there is more variability in the progression, perhaps with waxing and waning or with intermittent immune attacks (3). Moreover, the disease may be a consequence of imbalance between the immune system and the ability of the pancreatic beta cell to withstand attack (4).

A genetic basis for the disease in association with human leukocyte antigen (HLA) was first described in the early 1970s (5). Subsequently, that relationship has been extensively characterized (6,7,8,9), with both Class II (9,10) and Class I HLA (11) contributing to genetic susceptibility, and even the identification of protective HLA haplotypes (12). Although multiple other potential genes have been identified as possible contributors to type 1 diabetes (13,14), the HLA region remains the major contributor to genetic predisposition (15). Indeed, based on a study of the general population of Denver newborns, children born with the high-risk genotype HLA-DR3/4-DQ8 comprise almost 50% of children who develop

anti-islet autoimmunity by age 5 years (16). In addition, the cumulative burden of non-major histocompatibility complex (MHC) susceptibility genes may play a role in determining the rate of disease progression. Genetic factors associated with type 1 diabetes are described in detail in Chapter 12 *Genetics of Type 1 Diabetes*.

In genetically susceptible individuals, the disease process eventuating in type 1 diabetes likely is initiated by an environmental trigger (17,18). It is unclear whether such a trigger is an infectious agent, such as an enterovirus, a dietary factor, alteration of the intestinal microbiome, or some other factor. Moreover, the association between environmental factors and

the course of the disease is complicated by observations that not only initiation of the disease process, but also the rate of progression to clinical onset, may be affected by environmental determinants and that metabolic decompensation at disease onset may be a consequence of another unrelated or nonspecific environmental event. Ongoing observational cohort studies, such as The Environmental Determinants of Diabetes in the Young (TEDDY) study (19,20), are designed to ascertain environmental determinants that may trigger islet autoimmunity and either speed up or slow down the progression to clinical onset in subjects with persistent islet autoimmunity. Please see Chapter 11 *Risk Factors for Type 1 Diabetes* for more discussion of putative environmental triggers of type 1 diabetes.

The type 1 diabetes immune response is initiated by antigen presentation and then mediated by T lymphocytes (21,22), resulting in a lymphocytic inflammatory response in pancreatic islets that has been called insulitis (23). It appears to involve an autoreactive response by both effector CD4 (24) and cytotoxic CD8 (25) T lymphocytes. These have the capacity to mediate damage both via cytokine effects (possibly involving such cytokines as interleukin-1 [IL-1] and tumor necrosis factor alpha [TNF-α]) or direct cytotoxic T lymphocyte-mediated lysis. This initial immune response, with continued lysis, creates the potential of a vicious cycle of inflammation, which also may engender secondary and tertiary immune responses that contribute to the impairment of beta cell function and potential destruction of beta cells (3,4,21). This insidious process evolves over a variable amount of time—even many years in some individuals. The eventual overt manifestation of clinical symptoms becomes apparent only when most beta cells have lost function and many may have been destroyed.

The initial laboratory manifestation of this beta cell injury is seroconversion, i.e., the appearance of diabetes-related autoantibodies. These antibodies are generally thought not to mediate beta cell injury but rather to be markers of such

FIGURE 37.1. Natural History of Type 1 Diabetes, 1986
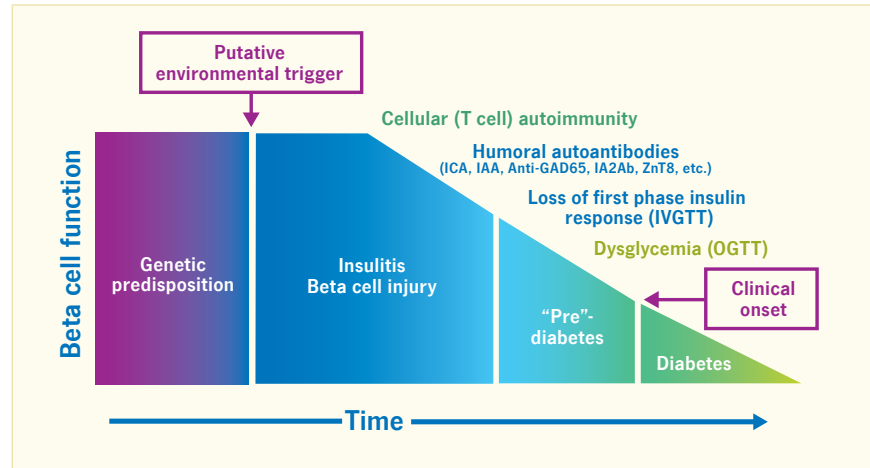
A model of the natural history of type 1 diabetes, as proposed by Dr. George S. Eisenbarth in 1986. GAD65, glutamic acid decarboxylase 65 kD; IA2Ab, islet antibody-2; IAA, insulin autoantibodies; ICA, islet cell antibodies; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test; ZnT8, zinc transporter 8.
SOURCE: Adapted from Reference 1, copyright © 1986 Massachusetts Medical Society, reprinted with permission

FIGURE 37.2. Stages of Type 1 Diabetes, 2015
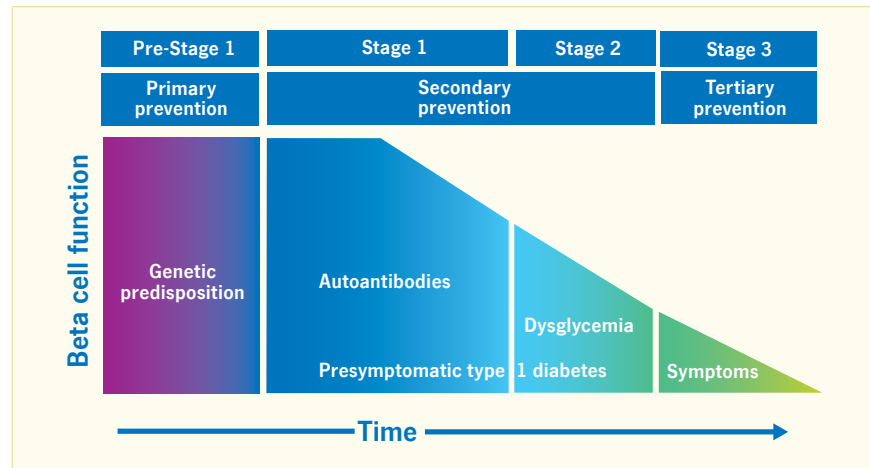
A model of type 1 diabetes staging, as proposed in a joint scientific statement of the JDRF, Endocrine Society, and American Diabetes Association in 2015.
SOURCE: Adapted from Reference 32, copyright © 2015 American Diabetes Association, reprinted with permission from the American Diabetes Association

injury. Diabetes-related autoantibodies were first described in the early 1970s, when islet cell antibodies (ICA) were identified by immunofluorescence (26). Subsequently, additional antibodies were identified with specific antigen targets, including insulin autoantibodies (IAA), antibodies to glutamic acid decarboxylase (GAD), antibodies to an aborted tyrosine phosphatase, which has been called islet antibody-2 (IA2), and antibodies to the zinc transporter (ZnT8) (27), all of which are components of beta cells.

Seroconversion is an important marker of the type 1 diabetes disease process. Indeed, in longitudinal studies of birth cohorts identified by genetic screening, such as DAISY (Diabetes AutoImmunity Study in the Young) (28), BABYDIAB (29), and DIPP (DIabetes Prediction and Prevention study) (30), if two or more antibodies appear, there is near certain progression to type 1 diabetes over the next two decades (31). This finding has led to a new classification of type 1 diabetes (Figure 37.2), in which the presence of

two or more antibodies defines Stage 1 of type 1 diabetes (32).

During further evolution of the disease, progressive metabolic changes are observable (33). Lack of beta cell sensitivity to glucose, i.e., failure of the beta cell to recognize glucose and appropriately secrete insulin, is an early defect (34), similar to that seen in type 2 diabetes (35). This may be manifested by loss of first phase insulin response to intravenous glucose (36) and dysglycemia (abnormal glucose levels not reaching the threshold for clinical diagnosis), which defines Stage 2 type 1 diabetes. Ultimately, there is progression to clinical type 1 diabetes (37), now also called Stage 3 (32). A risk score, taking into account several of these metabolic changes, has been developed (38) and validated (39). After the clinical onset of type 1 diabetes, there is further progressive decline of beta cell function (40).

One would expect that if type 1 diabetes is an immunologically mediated disease, then immune intervention should alter the natural history of the disease and potentially abrogate the clinical syndrome. This has certainly been the case in animal models of type 1 diabetes (41,42,43). The first reported attempt at immune intervention in type 1 diabetes was in the late 1970s in a handful of subjects (44). In the 1980s, a number of small trials were conducted with a variety of immunologic agents (45,46). Since then, initially stimulated by a provocative pilot study with cyclosporine (47), a large number of studies have been conducted, mostly in recent-onset type 1 diabetes in an attempt to interdict the disease process and preserve beta cell function (48,49). A few studies have been conducted prior to any evidence of autoimmunity (primary prevention) or after the development of diabetes-related autoantibodies (secondary prevention) (50). The goal of such primary and secondary interventions is to arrest the immune process and, thus, prevent or delay clinical disease.

Table 37.1 lists both completed and ongoing primary and secondary prevention trials. Table 37.2 lists a large number of contemporary intervention trials in subjects with clinical Stage 3 type 1 diabetes, mostly in recent-onset subjects, but some in established disease. Most studies listed are randomized controlled clinical trials, although a few pilot studies of significance are included.

PRIMARY PREVENTION TRIALS

Primary prevention trials (Table 37.1) have been conducted in birth cohorts identified by genetic screening, with the interventions initiated at a time when there are neither signs of autoimmunity nor metabolic impairment. Since there is uncertainty as to whether those infants identified by genetic screening will progress to type 1 diabetes, any interventions tested must be extremely safe. As a consequence, virtually all primary prevention trials to date have involved dietary interventions directed at putative environmental triggers of type 1 diabetes (51,52,53,54,55,56).

A meta-analysis had demonstrated a correlation between onset of type 1 diabetes and either early introduction of cow's milk formula or a short period of breastfeeding (57). Consequently, two studies evaluated whether at the time of weaning, replacement of breast milk with a formula based on casein hydrolysate rather than conventional cow's milk-based formula could reduce the development of autoimmunity (51,52). Eligible infants had HLA-conferred susceptibility to type 1 diabetes and at least one family member with type 1 diabetes. A pilot study in Finland enrolled 230 infants (51). The investigators reported that the group assigned to casein hydrolysate formula had a reduced risk of development of beta cell autoimmunity (appearance of one or more antibodies) (hazard ratio [HR] 0.54, 95% confidence interval [CI] 0.29–0.95; HR adjusted for observed difference in duration of exposure to study formula 0.51, 95% CI 0.28–0.91) (51). The larger Trial to Reduce IDDM in the Genetically at Risk (TRIGR) study, a multinational trial involving 77 centers in 15 countries, registered over 5,000 newborns and randomized 2,159 newborns with risk genotypes (approximately 45% of those screened) (52). After 7 years, the TRIGR Study Group found no difference in the rate of appearance of diabetes autoantibodies (52). In the group assigned to casein hydrolysate formula, 13.4% had two or more islet autoantibodies versus 11.4% among those randomized to the conventional formula (unadjusted HR 1.21, 95% CI 0.94–1.54). When the hazard ratio was adjusted for HLA risk, duration of breastfeeding, vitamin D use, study formula duration and consumption, and region of the world, it was 1.23 (95% CI 0.96–1.58). Nonetheless, TRIGR is continuing follow-up because it was designed with a primary outcome of the development of type 1 diabetes by age 10 years.

To evaluate whether bovine insulin might be the component of cow's milk that serves as a trigger for type 1 diabetes, the Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes (FINDIA) compared three formulas: cow's milk formula (control), whey-based hydrolyzed formula, or whey-based FINDIA formula essentially free of bovine insulin, whenever breast milk was not available during the first 6 months of life (53). Of 5,003 infants screened, 1,113 were found eligible, 1,104 were randomized, and 908 provided at least one follow-up sample. By age 3 years, the group assigned to the FINDIA formula had a reduced risk of development of beta cell autoimmunity, defined as the appearance of one or more antibodies (in the intention-to-treat analysis, odds ratio [OR] 0.39, 95% CI 0.17–0.91, p=0.03; in the actual treatment-received analysis, OR 0.23, 95% CI 0.08–0.69, p<0.01, in the FINDIA group when compared with the cow's milk formula group) (53).

TABLE 37.1. Trials for Primary and Secondary Prevention of Type 1 Diabetes

STUDY NAME (REF.)	INTERVENTION	NUMBER RANDOMIZED	ACTIVE: PLACEBO	AGE RANGE (YEARS)	PRIMARY OUTCOME	HAZARD RATIO (95% CI)	P-VALUE
Primary prevention studies—completed							
Finnish TRIGR Pilot (51)	Casein hydrolysate formula	230	1:1	birth	Autoantibodies	0.54 (0.29–0.95)	0.005
TRIGR (52)	Casein hydrolysate formula	2,159	1:1	birth	Autoantibodies	1.21 (0.94–1.54)	0.14
FINDIA (53)	Insulin-free whey based formula	1,104	1:1:1	birth	Autoantibodies	OR 0.39 (0.17–0.91)	0.03
BABYDIET (54)	Gluten-free diet	150	1:1	birth	Autoantibodies	1.3 (0.6–3.0)	0.6
NIP Pilot Trial (55)	Docosahexaenoic acid	98	1:1	birth	Cytokines		NS
Canadian Vitamin D Pilot Trial (56)	Supplemental vitamin D	9	1:1	birth	25-OH-D levels		NA
Pre-POINT (59)	Oral insulin	25	1.5:1	2–7	Safety	No issues	NA
Primary prevention studies—ongoing*							
TRIGR (52)	Casein hydrolysate formula	2,159	1:1	birth	Diagnosis of type 1 diabetes	†	†
Secondary prevention studies—completed							
DENIS (61)	Nicotinamide	55	1:1	3–12	Diagnosis of type 1 diabetes	0.79 (0.25–3.38)	0.97
ENDIT (62)	Nicotinamide	552	1:1	3–40	Diagnosis of type 1 diabetes	1.07 (0.78–1.45)	0.69
DPT-1 Parenteral Insulin (63)	Injected insulin	339	1:1	4–45	Diagnosis of type 1 diabetes	0.96 (0.69–1.34)	0.80
DPT-1 Oral Insulin (64,65,66)	Oral insulin	372	1:1	3–45	Diagnosis of type 1 diabetes	0.76 (0.51–1.14)	0.189
Belgian Parenteral Insulin (67)	Injected insulin	50	1:1	5–40	Diagnosis of type 1 diabetes	NA	0.97
DIPP birth cohort (68)	Nasal insulin	224	1:1	1–5	Diagnosis of type 1 diabetes	1.14 (0.73–1.77)	0.55
DIPP sibling cohort (68)	Nasal insulin	40	1:1	4–11	Diagnosis of type 1 diabetes	1.93 (0.56–6.77)	0.30
INIT-1 Pilot Safety Study (69)	Nasal insulin	38	Cross-over	5–33	Safety	NA	NA
Secondary prevention studies—ongoing*							
INIT-II (70)	Nasal insulin	110	1:1	4–30	Diagnosis of type 1 diabetes	†	†
TrialNet Oral Insulin (71)	Oral insulin	393	1:1	3–45	Diagnosis of type 1 diabetes	†	†
DIAPREV-IT (72)	Glutamic acid decarboxylase	50	1:1	4–18	Diagnosis of type 1 diabetes	†	†
TrialNet Teplizumab (73)	Anti CD3 - Teplizumab	71	1:1	8–45	Diagnosis of type 1 diabetes	†	†
TrialNet Abatacept (74)	Abatacept	Enrolling – 132 of 206*	1:1	6–45	Diagnosis of type 1 diabetes	†	†

25-OH-D, 25-hydroxyvitamin D; CI, confidence interval; DENIS, German (Deutsch) Nicotinamide Diabetes Intervention Study; DIAPREV-IT, Diabetes Prevention - Immune Tolerance study; DIPP, Diabetes Prediction and Prevention Study; 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; NA, not applicable; NIP, Nutritional Intervention to Prevent Type 1 Diabetes; NS, nonsignificant; OR, odds ratio; Pre-POINT, Primary Oral Insulin Therapy Study; TRIGR, Trial to Reduce the Incidence of Diabetes in the Genetically at Risk.

* As of January 2017

† Data are not available as of January 2017.

SOURCE: References are listed within the table.

The BABYDIET study, a randomized controlled trial, evaluated whether delayed exposure to gluten reduces the risk of diabetes autoimmunity (54). The rationale for the study was based on the investigators' earlier observation of increased risk of islet autoimmunity in children who are exposed to gluten early in life (58). The trial randomized 150 infants with a first-degree relative with type 1 diabetes and an HLA genotype consistent with type 1 diabetes risk. They were assigned either to first gluten exposure at age 6 months (control group) or at age 12 months (late-exposure group) and were followed every 3 months until age 3 years and yearly thereafter. BABYDIET found that delaying gluten exposure until the age of 12 months is safe but does not substantially reduce the risk for islet autoimmunity (3-year risk: 12% vs. 13%, p=0.6) (54).

The TrialNet Nutritional Intervention to Prevent (NIP) Type 1 Diabetes Pilot Trial assessed the feasibility of implementing a study to determine the effect of nutritional supplements with the omega-3 fatty acid docosahexaenoic acid (DHA), which has anti-inflammatory effects, during the last trimester of pregnancy and the first few years of life (55). NIP found that supplementation of infant diets with DHA was safe and resulted in an increased level of DHA in infant erythrocytes but did not find consistent changes in inflammatory cytokines (55).

Based on putative observations that vitamin D may be protective against type 1 diabetes, a group of Canadian investigators showed in a small pilot study that it was possible to recruit babies from the general population for identification of HLA-associated risk status followed by enrollment by age 1 month to a randomized controlled prevention trial of vitamin D supplementation (56). Therefore, they have proposed a nationwide study (in Canada) to evaluate the hypothesis that vitamin D supplementation can decrease the risk of islet autoimmunity and type 1 diabetes.

The Primary Oral Insulin Therapy (Pre-POINT) study was a pilot safety study of the use of oral insulin in children age 2–7 years at risk of developing type 1 diabetes (59). The study found that daily oral administration of 67.5 mg insulin is safe and can actively engage the immune system with features of immune regulation in children who are genetically at risk of developing type 1 diabetes. Consequently, a larger randomized controlled trial has been initiated (60).

It would seem desirable to conduct more studies in those with genetic predisposition, particularly if a safe vaccine-type approach can be studied. This might involve an antigen-based vaccine (such as that used in the Pre-POINT study) or a vaccine directed against potential viral triggers of disease. In order to conduct such trials, it will be necessary to screen various populations at or shortly after birth, looking for high-risk genetic predisposition. Theoretically, if such trials resulted in prevention of type 1 diabetes, this would change public health practice, leading to routine screening at birth for high genetic risk of type 1 diabetes. Further, if the intervention were totally safe, it could ultimately be included in routine neonatal or infant vaccination programs.

SECONDARY PREVENTION TRIALS

Secondary prevention trials (Table 37.1) are those conducted in individuals with Stage 1 (autoantibodies alone) or Stage 2 type 1 diabetes (autoantibodies and metabolic dysfunction) (Figure 37.2) (32). Most of these studies have been conducted in individuals, generally first- or second-degree relatives of people with type 1 diabetes, who initially have been identified by screening for diabetes-associated autoantibodies. Because not all of those with antibodies will progress to type 1 diabetes, selection of interventions to be tested has been done cautiously. Indeed, all completed secondary prevention studies have used either nicotinamide (a water soluble vitamin [B6] derived from nicotinic acid) or insulin given in one form or another (61,62,63,64,65,66,67,68,69). Ongoing secondary prevention trials are either antigen-based—using insulin (nasal (70) or oral (71)) or GAD (72)—or use immunomodulatory therapies that have previously been found to be relatively safe and with beneficial effects on beta cell function in tertiary prevention studies in recent-onset type 1 diabetes, namely teplizumab (73) and abatacept (74).

NICOTINAMIDE TRIALS

Nicotinamide, a water-soluble vitamin, had been shown to prevent diabetes in animal models and was asserted to have beneficial effect in school children. Consequently, two studies evaluated the effects of nicotinamide in at-risk relatives of individuals with type 1 diabetes: the German (Deutsch) Nicotinamide Diabetes Intervention Study (DENIS) (61) and the European Nicotinamide Diabetes Intervention Trial (ENDIT) (62). Both were randomized placebo-controlled trials. DENIS randomized 55 relatives age 3–12 years and used a sequential interim analysis design, which provided a 10% probability of a type II error against a reduction of the cumulative diabetes incidence at 3 years from 30% to 6% by nicotinamide. The trial was terminated, after 11 cases of diabetes, when it failed to achieve that endpoint (p=0.97) (61). ENDIT screened over 35,000 relatives age 3–40 years and randomized 552 individuals to nicotinamide or placebo. By confining recruitment to ICA-positive, first-degree relatives of individuals in whom type 1 diabetes had onset at age <20 years, the relatives who screened positive were projected to have a 5-year risk of type 1 diabetes of 40%. During 4 years of follow-up, the rate of development of type 1 diabetes was nearly identical in both the nicotinamide and placebo groups, with an unadjusted hazard ratio of 1.07 (95% CI 0.78–1.45, p=0.69) (62). Thus, in these two studies, nicotinamide failed to delay the development of type 1 diabetes.

INSULIN TRIALS

The Diabetes Prevention Trial-Type 1 (DPT-1) Study Group conducted two studies concomitantly: (1) the DPT-1 Parenteral Insulin Trial (63) evaluated injected (parenteral) insulin in individuals with a projected 5-year risk of type 1 diabetes of at least 50% (who had Stage 2 type 1 diabetes) and (2) the DPT-1 Oral Insulin Trial (64) evaluated oral insulin in individuals with a projected 5-year risk of type 1 diabetes of 25%–50% (who had Stage 1 type 1 diabetes). DPT-1 screened over 100,000 relatives of patients with type 1 diabetes for ICA by immunofluo-rescence and randomized 339 and 372 subjects, respectively, in the two trials.

Eligibility for the DPT-1 Parenteral Insulin Trial required, in addition to antibodies, evidence of decreased metabolic function, manifested by either reduced first phase insulin response to intravenous glucose or glucose intolerance during an oral glucose tolerance test, thus meeting the criteria for Stage 2 type 1 diabetes. The experimental intervention was two daily injections of long-acting ultralente insulin, plus a 96-hour continuous intravenous insulin infusion at baseline and annually thereafter. The randomized control group was closely observed but did not receive placebo. The rate of development of diabetes was the same in both the treated group and the control group (HR 0.96, 95% CI 0.69–1.34, p=0.80) (63). The DPT-1 Parenteral Insulin Trial found that the actual 5-year rate of developing type 1 diabetes was 65%, greater than the projected rate of at least 50%.

Eligibility for the DPT-1 Oral Insulin Trial required, in addition to ICA, IAA, intact first phase insulin response to intravenous glucose, and normal glucose tolerance, thus meeting the criteria for Stage 1 type 1 diabetes. Randomized subjects received either oral insulin or matched placebo taken daily. The rate of development of diabetes was the same in both groups (HR 0.76, 95% CI 0.51–1.14, p=0.189) (64). The DPT-1 Oral Insulin Trial found that the actual 5-year rate of developing type 1 diabetes was 35%, within the projected range of 25%–50%. In a *post hoc* analysis,

a subgroup (individuals with higher IAA titers at baseline) was identified in which oral insulin appeared to have a beneficial effect. This subgroup had a projected delay of 4.5–5 years in onset of type 1 diabetes if baseline IAA titer was >80 nU/mL (64) and a projected delay of 10 years if baseline IAA titer was >300 nU/mL (65). Further follow-up of the DPT-1 oral insulin cohort showed that effects were maintained after administration of oral insulin was ceased (66). Because the subgroup with a potential beneficial effect was identified in a *post hoc* analysis, an ongoing trial conducted by Type 1 Diabetes TrialNet is examining oral insulin in subjects similar to those in the subgroup with higher titer IAA (71).

The Belgian Diabetes Registry also evaluated whether parenteral insulin might delay the development of type 1 diabetes (67). In this study, the experimental group received regular insulin twice daily before the most carbohydrate-rich meals, and the randomized control group was closely observed but did not receive placebo. Fifty subjects were randomized—25 each to treatment and control. Eligible subjects were age 5–40 years, with IA2 antibodies and normal oral glucose tolerance, thus meeting the criteria of Stage 1 type 1 diabetes. There was no difference in diabetes-free survival between the two groups (p=0.97), with 5-year progression of 44% in the treated group and 49% in the control group.

The DIPP study was conducted in Finland among newborns from the general population (i.e., without relatives with type 1 diabetes) with high-risk HLA-DQB1 susceptibility alleles for type 1 diabetes (68). Cord blood samples from 116,720 consecutively born infants were screened, which identified 17,397 with high or moderate genetic risk, of whom 10,577 participated in a prospective study with serial follow-up for presence of diabetes autoantibodies. The intervention study required at least two antibodies in two consecutive samples (Stage 1 type 1 diabetes); of 328 subjects who met that criteria, 224 were randomized to receive either intranasal insulin or placebo. DIPP

also screened siblings of those infants and followed those siblings who also had increased genetic risk; of 52 siblings who met enrollment criteria, 40 were randomized to receive intranasal insulin or placebo. During follow-up, within each of the cohorts (infants and siblings), the rate of progression to type 1 diabetes was the same in the intranasal insulin group and the placebo group (68).

Another study, conducted in Australia, the Intranasal Insulin Trial (INIT 1), used a double-blind crossover design to eval-uate safety of intranasal insulin (69). The study included 38 subjects at risk of type 1 diabetes, who were treated with either intranasal insulin or placebo, daily for 10 days and then 2 days per week for 6 months, after which they were crossed over to the other treatment. There was no acceleration of onset of type 1 diabetes nor were there other adverse outcomes. Intranasal insulin was associated with an increase in antibody and a decrease in T cell responses to insulin. Since there were no safety issues, the ongoing Intranasal Insulin Trial-II (INIT II), under the auspices of the Diabetes Vaccine Development Centre (DVDC) in Australia, is evaluating whether intranasal insulin can delay or prevent the onset of type 1 diabetes (70).

OTHER ONGOING SECONDARY PREVENTION TRIALS

As noted in Table 37.1, other ongoing secondary prevention trials include the Diabetes Prevention - Immune Tolerance study (DIAPREV-IT) with a GAD vaccine (72) and Type 1 Diabetes TrialNet studies using teplizumab (73) and abatacept (74). The enrollment criteria for three ongoing TrialNet studies are different. Eligibility for the TrialNet oral insulin study (71) requires at least two antibodies, one of which is IAA, intact first phase insulin response to intravenous glucose, and normal glucose tolerance. Eligibility for the TrialNet abatacept study (74) requires at least two antibodies, one of which is not IAA, and normal glucose tolerance. Eligibility for the TrialNet teplizumab study (73) requires at least one antibody and dysglycemia during an oral glucose tolerance test.

SCREENING AND ENROLLMENT FOR SECONDARY PREVENTION TRIALS

Secondary prevention trials involve screening of relatives of people with type 1 diabetes and enrollment of those with early markers of disease, either autoantibodies alone (Stage 1) or autoantibodies and metabolic dysfunction (Stage 2). In cross-sectional screening of relatives for autoantibodies in DPT-1 and TrialNet, <5% of relatives are found to have autoantibodies. Although this rate is tenfold to twentyfold higher than would be seen in the general population of the United States, it still means that to enroll secondary prevention trials, large numbers of subjects need to be screened. For example, DPT-1 screened over 100,000 relatives to enroll a total of 711 subjects in the two arms of that study (parenteral insulin and oral insulin).

TERTIARY PREVENTION TRIALS

Tertiary prevention trials (Table 37.2) have been conducted in subjects with Stage 3 clinical type 1 diabetes (i.e., classic symptomatic type 1 diabetes requiring insulin therapy) (Figure 37.2) (32), mostly recent onset, but some in established disease. As noted, there were many early pilot trials with a variety of immune interventions (45,46) that will not be discussed here. Rather, this discussion is confined to randomized controlled trials and studies that have either tested contemporary immunologic approaches or ones that offer special insights.

EARLY INTERVENTION STUDIES
Cyclosporine

A pilot study by Stiller *et al.* (47), reported in 1984, used cyclosporine, an immunosuppressive agent targeting T lymphocytes, which served to stimulate the field, including the conduct of a number of cyclosporine studies (75,76,77,78,79,80). Two large randomized controlled trials compared "remission" rates with cyclosporine versus placebo in subjects with new-onset Stage 3 type 1 diabetes (75,76). In the French study (75), "complete remission" was defined as good metabolic control (aiming at fasting blood glucose <140 mg/dL [<7.77 mmol/L], postprandial blood glucose <200 mg/dL [<11.10 mmol/L], and glycosylated hemoglobin [A1c] <7.5% [<58 mmol/mol]) in the absence of insulin treatment. "Partial remission" was defined by the same metabolic criteria obtained with <0.25 units/kg per day of insulin. In the Canadian-European study (76), the same metabolic targets were used, but "remission" also required a stimulated C-peptide level >0.6 nmol/L or a non-insulin requiring (NIR) state. Doses of cyclosporine were progressively lowered and stopped after a period of time if remission was not achieved.

Both studies showed a greater proportion of subjects in remission with cyclosporine than with placebo, but the rate of remission progressively declined in both groups during the 1-year course of the study. Two smaller studies, in Miami (77) and Denver (78), also were conducted. The Miami study showed a slower rate of decline of stimulated C-peptide with cyclosporine compared to placebo. The Denver study showed a slightly greater, but not statistically significant, difference in the rate of remission in the cyclosporine group than the placebo group. Meanwhile, buoyed by two randomized controlled trials showing the beneficial effects of cyclosporine, a French team initiated a study of cyclosporine in which all eligible subjects received the drug (79). In that study, 27 of 40 subjects (67.5%), all of whom were children, achieved remission. Enrollment was expanded, and subjects were followed for a protracted period of time, during which subjects lost their remission in spite of continued cyclosporine therapy (80). This lack of long-term benefit, coupled with the then-emerging recognition of cyclosporine side effects (particularly renal disease), led to virtual abandonment of this therapy in type 1 diabetes.

Azathioprine

In the same era, the mid-1980s, several studies were conducted with azathioprine, an immunomodulatory agent, in recent-onset Stage 3 type 1 diabetes (81,82,83). One study initiated therapy with a 10-week course of corticosteroids followed by 1 year of treatment with azathioprine and found better beta cell function at 1 year, as measured by peak C-peptide/glucose ratio, than in the randomized but untreated control group (81). Another, nonrandomized study gave alternate patients azathioprine and found that most azathioprine subjects achieved "remission," whereas only one comparison subject did (82). A third azathioprine study was a double-masked placebo-controlled study that enrolled 49 people age 2–20 years with newly diagnosed type 1 diabetes (83). This study found nearly equal rates of remission in both groups. Given the nonrandomized nature of the other studies and the side effects of azathioprine, further studies with azathioprine were not pursued.

Linomide

The immunomodulatory agent linomide (quinoline-3-carboxamide), thought to activate or modulate regulatory T lymphocytes, was evaluated in a randomized placebo-controlled trial in 63 subjects age 10–20 years with recent-onset Stage 3 type 1 diabetes (84). Subjects were treated for 1 year, and beta cell function was evaluated by glucagon-stimulated C-peptide. Although the initial analysis suggested no difference between groups, when the analysis was confined to those with residual C-peptide at baseline (40 of 63 subjects), a beneficial effect was observed. Although side effects were minimal, the manufacturer did not continue development of linomide, and thus, this was not further pursued.

Bacille Calmette-Guerin Vaccine

Two double-masked placebo-controlled trials in the 1990s evaluated the effects of BCG (bacille Calmette-Guerin) vaccine, an immune regulatory agent that showed benefit in animal models, in subjects with recent-onset Stage 3 type 1 diabetes (85,86). One, conducted in Alberta, Canada (85), enrolled 26 subjects with mean age 13 years, while the other, conducted in Colorado and Massachusetts (86), enrolled 94 subjects

TABLE 37.2. Intervention Studies in Recent-Onset Type 1 Diabetes

STUDY NAME (REF.)	INTERVENTION	NUMBER RANDOMIZED	ACTIVE: PLACEBO	AGE RANGE (YEARS)	PRIMARY OUTCOME	TIME OF PRIMARY OUTCOME	OUTCOME	P-VALUE
Early intervention studies (1970–2000)								
French Cyclosporine (75)	Cyclosporine	122	1:1	15–40	Remission	9 months	24.1% vs. 5.8%	<0.01
Canadian-European Cyclosporine (76)	Cyclosporine	188	1:1	9–35	C-peptide ≥0.6 nmol/L or non-insulin requiring	1 year	33.0% vs. 20.7%	0.004
Miami Cyclosporine Trial (77)	Cyclosporine	23	1:1	9–38	MMTT C-peptide	1 year	Slower rate of decline	<0.05
Denver Cyclosporine Trial (78)	Cyclosporine	43	1:1	8–36	Remission	1 year	27.2% vs. 19.0%	NS
French Pediatric Cyclosporine (79,80)	Cyclosporine	40	All treated (no controls)	7–15	Remission	1 year	67.5%	NA
Azathioprine + Glucocorticoids (81)	Azathioprine and Prednisone	46	1:1	4–33	Peak C-peptide/ glucose ratio	1 year	44.1 vs. 14.2	<0.01
Azathioprine – Adults (82)	Azathioprine	24	1:1	15–50	Remission	1 year	53.8% vs. 9.1%	<0.05
Azathioprine – Children (83)	Azathioprine	49	1:1	2–20	Partial remission	1 year	17% vs. 16%	NS
Linomide French Trial (84)	Linomide	63	2:1	10–20	Glucagon-stimulated C-peptide	1 year	0.38 vs. 0.25 nmol/L	<0.05
BCG (85)	BCG vaccine	26	1:1	Mean 13	Glucagon-stimulated C-peptide	18 months	0.20 vs. 0.30 nmol/L	NS
BCG (86)	BCG vaccine	94	1:1	5–18	1°: Remission 2°: MMTT C-peptide	2 years	1°: 2.5% vs. 2.6% 2°: No diff	NS
French Oral Insulin (87)	Oral insulin (two doses: 2.5 mg, 7.5 mg)	131	1:1:1	7–40	Glucagon-stimulated C-peptide	1 year	0.39 vs. 0.37 vs. 0.33 nmol/L	NS
Italian Oral Insulin (88)	Oral insulin (5 mg)	82	1:1	5–36	Fasting C-peptide	1 year	0.17 vs. 0.22 nmol/L	NS
U.S. Oral Insulin (89)	Oral insulin (two doses: 1 mg, 10 mg)	191	1:1:1	5–80	Loss of C-peptide	6–36 months	*	*
Anti-CD5 Pilot (90)	Anti-CD5 immunotoxin	15	All treated (no controls)	17–40	MMTT C-peptide	1 year	Slope not different from zero	NA
Anti-CD3 intervention studies								
Herold Anti-CD3 (91,92)	Teplizumab	24	1:1	7–30	MMTT C-peptide	1 year	114.2 vs. 66.7 nmol/L	<0.01
Keymeulen Anti-CD3 (93,94)	Otelixizumab	80	1:1	12–39	C-peptide after clamp	1 year	0.8 vs. 0.6 nmol/L/min	<0.01
Protégé (95,96)	Teplizumab (three treatment regimens)	516	2:1:1:1	8–35	Insulin <0.5 u/kg and A1c <6.5%	1 year	19.8%, 13.7%, 20.8%, 20.4%	NS
Protégé Encore (97)	Teplizumab	256	NA	8–35	Insulin <0.5 u/kg and A1c <6.5%	1 year	NA	NA
DEFEND-1 (98)	Otelixizumab	281	2:1	12–45	MMTT C-peptide	1 year	-0.20 vs. -0.22 nmol/L	NS

Table 37.2 continues on the next page.

TABLE 37.2. (continued)

STUDY NAME (REF.)	INTERVENTION	NUMBER RANDOMIZED	ACTIVE: PLACEBO	AGE RANGE (YEARS)	PRIMARY OUTCOME	TIME OF PRIMARY OUTCOME	OUTCOME	P-VALUE
DEFEND-2 (99)	Otelixizumab	179	2:1	12–45	MMTT C-peptide	1 year	-0.23 vs. -0.13 nmol/L	NS
AbATE (ITN study) (100)	Teplizumab	83	2:1	8–29	MMTT C-peptide	2 years	-0.28 vs. -0.46 nmol/L	0.002
Delay (101)	Teplizumab	63	1:1	8–28	MMTT C-peptide	1 year	0.45 vs. 0.37 nmol/L	0.03
GAD intervention studies								
GAD Pilot (102)	GAD-Alum vaccine	70	1:1	10–18	Fasting C-peptide	15 months	-0.12 vs. -0.17 nmol/L	NS
GAD TrialNet (103)	GAD-Alum vaccine (two dose regimens)	145	1:1:1	3–45	MMTT C-peptide	1 year	0.41 vs. 0.38 vs. 0.41 nmol/L	NS
GAD Europe (104)	GAD-Alum vaccine (two dose regimens)	334	1:1:1	10–20	MMTT C-peptide	1 year	All approx. -0.33 nmol/L	NS
GAD U.S. (DIAPREVENT) (105)	GAD-Alum vaccine (two dose regimens)	328	1:1:1	10–20	MMTT C-peptide	1 year	NA	NS
DiaPep277 intervention studies								
DiaPep – Israeli adults (106,107)	DiaPep277 peptide	35	1:1	16–55	Glucagon-stimulated C-peptide	10 months	0.93 vs. 0.26 nmol/L	0.039
DiaPep – Israeli children (108)	DiaPep277 peptide	30	1:1	7–14	MMTT C-peptide	1 year	0.21 vs. 0.17 nmol/L	NS
DiaPep – Belgian adults (109)	DiaPep277 peptide (three doses: 0.2 mg, 1.0 mg, 2.5 mg)	48	1:1:1:1	18–45	Glucagon-stimulated C-peptide	1 year	-2.5, -2.5, -0.5, -5.0 nmol/min/L	0.03† 2.5 mg dose
DiaPep – Europe adults (110)	DiaPep277 peptide (three doses: 0.2 mg, 1.0 mg, 2.5 mg)	50	1:1:1:1	16–44	Glucagon-stimulated C-peptide	18 months	Similar change	NS
DiaPep – Europe children (110)	DiaPep277 peptide (two doses: 0.2 mg, 1.0 mg)	49	1:1:1	4–15	Glucagon-stimulated C-peptide	18 months	Similar change	NS
DiaPep – Phase III (111,112)	DiaPep277 peptide	457	1:1	16–45	Glucagon-stimulated C-peptide	24 months	‡	‡
Other TrialNet intervention studies								
MMF-DZB (115)	Mycophenolate mofetil (MMF) and daclizumab (DZB)	126	1:1:1	8–45	MMTT C-peptide	2 years	0.25 vs. 0.28 vs. 0.27 nmol/L	NS
Anti-CD20 (116,117)	Anti-CD20 Rituximab	87	2:1	8–40	MMTT C-peptide	1 year	0.56 vs. 0.47 nmol/L	0.03
Abatacept (118,119)	Abatacept	112	2:1	6–45	MMTT C-peptide	2 years	0.378 vs. 0.238 nmol/L	0.0029
Canakinumab (120)	Anti-IL1β canakinumab	71	2:1	6–45	MMTT C-peptide	1 year	Diff = 0.01 nmol/L	NS
Other ITN intervention studies								
START Thymoglobulin (121)	Thymoglobulin	58	2:1	12–35	MMTT C-peptide	1 year	-0.195 vs. -0.239 nmol/L	NS

Table 37.2 continues on the next page.

TABLE 37.2. (continued)

STUDY NAME (REF.)	INTERVENTION	NUMBER RANDOMIZED	ACTIVE: PLACEBO	AGE RANGE (YEARS)	PRIMARY OUTCOME	TIME OF PRIMARY OUTCOME	OUTCOME	P-VALUE
T1DAL – Alefacept (122)	Alefacept	49	2:1	12–35	MMTT C-peptide: 1°: 2 hours 2°: 4 hours	1 year	1°: +0.015 vs. -0.115 nmol/L 2°: +0.015 vs. -0.156 nmol/L	0.065 0.019
Insulin B-Chain (124)	Insulin B-chain	12	1:1	18–35	MMTT C-peptide	1 year	Similar change	NS
IL-2 & Rapamycin Safety (125)	IL-2 and rapamycin	9	All treated (no controls)	20–36	MMTT C-peptide	1 year	43% decrease at 3 months	NA
Other recent intervention studies								
AIDA Anakinra Trial (120)	Anakinra	69	1:1	18–35	MMTT C-peptide	9 months	Diff =0.02 nmol/L	NS
Alpha-1 Antitrypsin (AAT) (126)	AAT	12	All treated (no controls)	12–39	MMTT C-peptide	1 year	*	NA
Altered peptide ligand (APL) (127)	B9-23 APL (three doses)	188	1:1:1:1	10–35	MMTT C-peptide	24 months	0.59, 0.57, 0.48, 0.54 nmol/L	NS
Plasmid-encoded proinsulin (128)	Plasmid-encoded proinsulin (four doses)	80	1:1:1:1:2	18–40	Safety and MMTT C-peptide	2 years	*	NS
Proinsulin peptide (129)	Proinsulin peptide (two doses)	48	1.5:1.5:1	21–53	Safety study	6 months	No safety issues	§
ATG – GCSF Trial (130)	Thymoglobulin and granulocyte colony-stimulating factor (GCSF)	25	2:1	12–45	MMTT C-peptide	1 year	0.74 vs. 0.43 nmol/L/min	0.05
DIATOR (133,134)	Atorvastatin	89	1:1	18–39	MMTT C-peptide	18 months	0.78 vs. 0.41 nmol/L	NS
Etanercept (135)	Etanercept	18	1:1	7–18	MMTT C-peptide	6 months	+39% vs. -20%	0.05
Low-Dose IL-2 Safety Trial (136)	IL-2 (three doses)	24	1:1:1:1	18–55	Treg number	60 days	Increased Tregs	NA
REPAIR-T1D (137)	Sitagliptin and lansoprazole	68	2:1	11–36	MMTT C-peptide	1 year	-229 vs. -253 pmol/L	NS
AHSCT + Profound Immunosuppression (138,139,140)	Cyclophosphamide, GCSF, ATG, AHSCT	23	All treated (no controls)	13–31	MMTT C-peptide	2 years	Increase from 225 to 785 ng/ml/2-hour	0.001
AHSCT + Profound Immunosuppression (141)	Cyclophosphamide, GCSF, ATG, AHSCT	65	All treated (no controls)	12–35	MMTT C-peptide	6 months	Increase from 0.54 to 1.22 ng/ml	0.0001
Ongoing intervention studies ‖								
ATG – GCSF Trial (132)	Thymoglobulin and GCSF	Enrolling Target: 84	1:1:1	12–45	MMTT C-peptide	1 year	¶	¶
EXTEND Trial (142)	Tocilizumab	Enrolling Target: 108	2:1	6–45	MMTT C-peptide	1 year	¶	¶
Otelixizumab Dose Ranging Trial (143)	Otelixizumab (four doses)	Enrolling Target: 40	1:1:1:1:1	16–27	Safety and MMTT C-peptide	2 years	¶	¶
Alpha-1 Antitrypsin (AAT) Trial (144)	AAT (two doses)	Enrolling Target: 192	1:1:1	8–25	Basal C-peptide	1 year	¶	¶
Alpha-1 Antitrypsin (AAT) Trial (145)	AAT (four regimens)	Enrolling Target: 75	1:1:1:1:1	12–35	MMTT C-peptide	1 year	¶	¶

Table 37.2 continues on the next page.

TABLE 37.2. (continued)

STUDY NAME (REF.)	INTERVENTION	NUMBER RANDOMIZED	ACTIVE: PLACEBO	AGE RANGE (YEARS)	PRIMARY OUTCOME	TIME OF PRIMARY OUTCOME	OUTCOME	P-VALUE
Ustekinumab Pilot (146)	Open label pilot ustekinumab (four doses)	Enrolling Target: 20	1:1:1:1	18–35	Safety	1 year	¶	¶
Imatinib Trial (147)	Imatinib	Enrolling Target: 66	2:1	18–45	MMTT C-peptide	1 year	¶	¶
Tauroursodeoxycholic Acid (TUDCA) Trial (148)	TUDCA	Enrolling Target: 20	1:1	18–45	MMTT C-peptide	18 months	¶	¶
DIABGAD (149)	GAD-Alum and vitamin D with/ without ibuprofen	Enrolling Target: 60	1:1:1:1	10–18	MMTT C-peptide	30 months	¶	¶
Proinsulin Peptide (150)	Proinsulin peptide (two dose regimens)	Enrolling Target: 24	1:1:1	18–40	Safety	3 years	¶	¶
Methyldopa (151)	Methyldopa	Enrolling Target: 50	All treated (no controls)	18–46	Inhibition of DQ8 antigen presentation; MMTT C-peptide	12 weeks	¶	¶
Low-Dose IL-2 (152)	IL-2	Enrolling Target: 24	1:1:1:1	7–12	Treg number	22 days	¶	¶

Conversions for A1c values are provided in *Diabetes in America Appendix 1 Conversions*. A1c, glycosylated hemoglobin; AAT, alpha-1 antitrypsin; AbATE, Autoimmunity-Blocking Antibody for Tolerance in Recently Diagnosed Type 1 Diabetes trial; AHSCT, autologous hematopoietic stem cell therapy; AIDA, Anti-Interleukin-1 in Diabetes Action; APL, altered peptide ligand; ATG, antithymocyte globulin; BCG, bacille Calmette-Guerin; DEFEND, Trial of Otelixizumab for Adults With Newly Diagnosed Type 1 Diabetes Mellitus (Autoimmune); DIABGAD, Trial to Preserve Insulin Secretion in Type 1 Diabetes Using GAD-Alum (Diamyd) in Combination With Vitamin D and Ibuprofen; DIAPREVENT, A Phase III Study to Investigate the Impact of Diamyd in Patients Newly Diagnosed With Type 1 Diabetes; DIATOR, Diabetes and Atorvastatin Trial; DZB, daclizumab; EXTEND, Tocilizumab in New Onset Type 1 Diabetes trial; GAD, glutamic acid decarboxylase; GCSF, granulocyte colony-stimulating factor; IL, interleukin; ITN, Immune Tolerance Network; MMF, mycophenolate mofetil; MMTT, mixed meal tolerance test; NA, not applicable; NS, nonsignificant; REPAIR-T1D, Combination Therapy With Sitagliptin and Lansoprazole to Restore Pancreatic Beta Cell Function in Recent-Onset Type 1 Diabetes trial; START, Study of Thymoglobulin to ARrest Type 1 diabetes; T1DAL, Inducing Remission in New Onset T1DM with Alefacept trial; TUDCA, tauroursodeoxycholic acid.
* Data are ambiguous.
† Outcome was significant in one of three dosages.
‡ The paper was retracted.
§ Randomized safety study
‖ As of January 2017
¶ Data are not yet available as of January 2017.

SOURCE: References are listed within the table.

age 5–18 years. Similar outcomes were seen in both studies, namely that there was no effect of BCG on preservation of beta cell function. Indeed, in both studies, there was a trend to greater decline of beta cell function in the BCG group than in the control group.

Oral Insulin

Three studies used oral insulin (in various doses) in recent-onset Stage 3 type 1 diabetes (87,88,89). In the French (87) and Italian (88) studies, no effect was seen on beta cell function. In the study conducted in the United States (89), retention of endogenous beta cell function was said to be dependent upon initial stimulated C-peptide response, age at diabetes onset, and numbers of specific islet cell autoantibodies found. The complex analysis did not permit a clear conclusion to be drawn, particularly in view of the two negative European studies.

Anti-CD5 Monoclonal Antibody

Using an anti-CD5 monoclonal antibody, which targets T lymphocytes, linked to ricin A-chain, a toxin, a small open-label dose-escalation pilot study was conducted in 15 subjects with recent-onset Stage 3 type 1 diabetes (90). With only 5 days of treatment, there appeared to be a slower than anticipated decline in beta cell function (tested by a mixed meal tolerance test [MMTT]) over 1 year, but in the absence of a control group, it was not possible to infer a beneficial effect. Nonetheless, the use of a monoclonal antibody directed at

T lymphocytes served to stimulate other investigations of monoclonal antibodies in type 1 diabetes.

ANTI-CD3 INTERVENTION STUDIES

Extensive studies have been conducted with two anti-CD3 monoclonal antibodies targeting T lymphocytes—teplizumab and otelixizumab—which are humanized Fc-mutated (Fc receptor [FcR] nonbinding) monoclonal antibodies. The first study reported was a small study involving only 12 treated subjects and 12 untreated comparison subjects (91). They received a single 14-day course of treatment with teplizumab within 6 weeks of diagnosis of Stage 3 type 1 diabetes and were found to have slower decline of beta cell function (by MMTT) at 1 year (91). In

these and an expanded group of subjects (total of 21 treated, 21 untreated), there was sustained improvement of beta cell function at 2 years (92). Meanwhile, the otelixizumab study was the first randomized placebo-controlled trial with an anti-CD3 monoclonal antibody (93). In it, 80 subjects age 12–39 years, within 4 weeks from diagnosis of Stage 3 type 1 diabetes, were randomized to a 6-day course of either otelixizumab or placebo and followed for 18 months. Beta cell function measured using a hyperglycemic clamp followed by glucagon stimulation was found to be better in the otelixizumab than the placebo group, particularly in subjects with higher baseline insulin secretory response (93). After 4 years of follow-up, although beta cell function was not measured, the otelixizumab group had lower insulin requirements despite similar glycemic control as measured by A1c (94). Thus, effects of a 6-day treatment course were evident 4 years later.

The results from these early Phase 2 studies with anti-CD3 treatment led to the initiation of Phase 3 clinical trials with both agents. However, the Phase 3 studies did not meet their primary outcome criteria. For teplizumab, the primary outcome was the combination of A1c <6.5% (<48 mmol/mol) and insulin dose <0.5 units/kg/day (95,96,97). This outcome measure was arbitrarily selected and highly criticized for a number of reasons. Moreover, by using a composite outcome that requires a subject to meet two criteria, the outcome became a dichotomous measure that dilutes the effect of two continuous variables—A1c and insulin dose. More importantly, when the conventional outcome measure of C-peptide was assessed, there was evidence of efficacy both at 1 year (95) and at 2 years (96) following two 14-day courses of teplizumab (at entry and at 26 weeks into the study). This was especially evident in subjects enrolled in the United States (who had lower A1c at entry and during study), in younger subjects (age 8–17 years), in subjects enrolled within 6 weeks of diagnosis, and in subjects with higher levels of C-peptide at entry (95). For otelixizumab, the Phase 3 studies used a

dose that was one-sixteenth (total of 3.1 mg over 8 days) that used in the positive Phase 2 study described in the previous paragraph (total of 48 mg), in an effort to avoid any side effects (98,99). Not only were side effects completely obviated, but beneficial effects were also obviated. This outcome highlights the challenge with significant dose reduction—all effective therapies are likely to have some side effects, and eliminating the side effects of a drug may also eliminate its potential benefits.

Two other studies with teplizumab are worth noting. In the Autoimmunity-Blocking Antibody for Tolerance in Recently Diagnosed Type 1 Diabetes (AbATE) Trial, conducted by the Immune Tolerance Network (ITN), there was demonstration of efficacy (100). More importantly, however, subjects could be divided into two groups—"responders" and "nonresponders" to treatment. Responders were those who maintained C-peptide better than the randomized, but untreated, comparison group at 24 months. This group, which constituted 45% of subjects treated with teplizumab, maintained beta cell function for 2 years, whereas the nonresponders lost beta cell function at a rate similar to the control group (100). In another teplizumab study, the Delay trial, subjects diagnosed with Stage 3 type 1 diabetes at least 4 but not more than 12 months before enrollment (thus "Delayed" compared to recent onset), were randomized to receive infusions of either teplizumab or placebo (101). There was a slowing in the decline of beta cell function in the group as a whole, driven by beneficial effect in those treated within 4–8 months of diagnosis, as the effects were not significant in the subgroup treated 9–12 months after diagnosis.

GAD INTERVENTION STUDIES
A vaccine, consisting of GAD with the adjuvant aluminum hydroxide (GAD-Alum), created much excitement on the basis of an initial report of a Phase 2 trial, in which there was claimed benefit, at least in those subjects enrolled early after diagnosis (102). However, this result was

not confirmed in a TrialNet study (103) nor in two Phase 3 trials conducted by the manufacturer (104,105).

DIAPEP277 INTERVENTION STUDIES
Several Phase 2 clinical trials were conducted using DiaPep277, a 24 amino acid peptide derived from heat shock protein 60 (106,107,108,109,110). The first of the Phase 2 trials appeared to have promising results (106,107), but the results from the other Phase 2 trials (108,109,110) were conflicting. A Phase 3 trial was reported and had inherently confusing results with improved C-peptide versus placebo during a glucagon-stimulated test, but no difference between groups with an MMTT (111,112). Subsequently, the papers describing this trial were retracted, because there may have been efforts to "manipulate the analyses to obtain a favorable result" (113,114).

OTHER TRIALNET INTERVENTION STUDIES
Type 1 Diabetes TrialNet has conducted four other studies with immunologic interventions in subjects with recently diagnosed type 1 diabetes. All studies enrolled subjects within 100 days of diagnosis of recent-onset Stage 3 type 1 diabetes and measured beta cell function by C-peptide in response to serial MMTTs.

One study evaluated the immunosuppressive agent mycophenolate mofetil, either alone or in combination with the anti-CD25 monoclonal antibody daclizumab, which targets the alpha chain of the IL-2 receptor expressed on T lymphocytes (115). The study enrolled 126 subjects age 8–45 years. It was stopped early by the Data and Safety Monitoring Board due to futility of the potential of seeing a beneficial treatment effect (115).

Another TrialNet study evaluated the anti-CD20 monoclonal antibody rituximab, which depletes B lymphocytes (116). In this study, 87 subjects age 8–40 years were randomized in a 2:1 design to receive either four weekly doses of rituximab or placebo. After 1 year, there was better maintenance of beta cell function

in the rituximab group than in the placebo group, although a progressive decline was observed in the rituximab group as well (116). Over 2 years, the rate of decline of C-peptide was parallel between groups, but shifted by 8.2 months in rituximab-treated subjects (117). Thus, the effect appeared to be transient, with no fundamental alteration of the disease process.

TrialNet also evaluated effects of abatacept (soluble CTLA4Ig), which binds to CD80 and CD86, the ligands for CD28, a co-stimulatory molecule on T lymphocytes (118). In this study, 112 subjects age 6–45 years were randomized in a 2:1 design to receive either monthly infusions of abatacept or placebo for 2 years. After those 2 years, there was better maintenance of beta cell function in the abatacept group than in the placebo group, although there was a progressive decline in the abatacept group as well (118). After therapy was stopped, subjects were followed for an additional year, with the abatacept group maintaining a difference from the placebo group; a progressive parallel rate of decline was observed in both groups, but shifted by 9.5 months in abatacept-treated subjects (119). Thus, the beneficial effect was sustained for at least 1 year after cessation of abatacept infusions or 3 years from the diagnosis of type 1 diabetes.

Another treatment strategy evaluated by TrialNet was antagonism of the cytokine IL-1, thought to be a key mediator of innate immunity, as it is a proinflammatory cytokine that recruits effector T lymphocytes in inflamed tissues and also has direct toxic effects on beta cells. In the TrialNet study, which used the anti-IL-1β monoclonal antibody canakinumab, 71 subjects age 6–45 years were randomized in a 2:1 design to receive either monthly subcutaneous injections of canakinumab or placebo for 1 year (120). No difference in beta cell function was observed between groups. The canakinumab study was reported together with another study examining antagonism of IL-1, using the human IL-1 receptor antagonist anakinra (120). For the anakinra

trial, 69 subjects age 18–35 years were randomized to receive either daily subcutaneous injections of anakinra or placebo for 9 months. With anakinra as well, there was no difference in beta cell function between groups. Thus, by itself, antagonism of IL-1 failed to show benefit.

OTHER IMMUNE TOLERANCE NETWORK INTERVENTION STUDIES

The ITN conducted a study evaluating thymoglobulin in recent-onset type 1 diabetes, the Study of Thymoglobulin to ARrest Type 1 diabetes (START) (121). In this study, 58 subjects age 12–35 years with recent-onset Stage 3 type 1 diabetes were randomized in a 2:1 design to receive either thymoglobulin (antithymocyte globulin [ATG]) or placebo over a course of 4 days. There was no between-group difference in beta cell function at 1 year. However, thymoglobulin resulted in generalized depletion of T lymphocytes rather than in the hoped-for specific depletion of effector memory T lymphocytes with preservation of regulatory T cells.

In another ITN study, the Inducing Remission in New-Onset Type 1 Diabetes with Alefacept (T1DAL) trial, alefacept was used to target memory T lymphocytes (122). In this trial, 49 subjects age 12–35 years, with recent-onset Stage 3 type 1 diabetes, were randomized in a 2:1 design to receive either alefacept or placebo, given as two 12-week courses of monthly intramuscular injections, separated by a 12-week hiatus. Because the manufacturer withdrew alefacept from production during the course of the trial, there was a smaller enrollment than planned. Beta cell function appeared to be preserved in the alefacept group, i.e., it did not decline over 12 months, but the results of the primary outcome——C-peptide during the first 2 hours of the MMTT——just missed statistical significance (p=0.065). In contrast, the secondary outcome——C-peptide during the full 4 hours of the MMTT——indicated a significant difference in beta cell function (p=0.019) (122). At 24 months, both the 4-hour and the 2-hour C-peptide levels were greater in the alefacept group than the placebo group (123). Thus, had the study been fully enrolled, the primary

outcome may have been met. Moreover, alefacept did appear to have a greater impact on central memory and effector memory T lymphocytes with sparing of naïve and regulatory T lymphocyte populations. Taken together, these findings suggest that targeting memory T lymphocytes may be an attractive immunomodulatory approach.

ITN also conducted two small pilot studies. One evaluated the safety of a vaccine using human insulin B-chain in incomplete Freund's adjuvant, administered as a single intramuscular injection (124). In this pilot safety study, 12 subjects age 18–35 years were randomized to receive either the vaccine or placebo. There were no safety issues. No difference in beta cell function was found, but there was suggestive evidence of generation of antigen-specific regulatory T lymphocytes.

The other ITN pilot study was an open label Phase 1 study using the combination of IL-2 and rapamycin (125). Nine subjects were enrolled, age 20–36 years, between 4 and 48 months from diagnosis of type 1 diabetes if they had a peak C-peptide of at least 0.4 nmol/L during an MMTT. The study was halted due to the reported acute decline in C-peptide during the first 3 months; although, without a comparison group and without much literature data on the rate of C-peptide decline in this time frame after diagnosis, it is not clear whether this was unusual. There appeared to be a subsequent recovery of C-peptide in four of the subjects. Although there was an increase in regulatory T lymphocytes, natural killer cells and eosinophils also increased, with no difference in effector T lymphocytes.

OTHER RECENT INTERVENTION STUDIES

A pilot Phase 1 safety study used alpha-1 antitrypsin (AAT), an anti-inflammatory agent that had beneficial effects in animal models (126). No safety issues were identified. The study showed that AAT was associated with a down-modulation of IL-1β, which may indicate potential benefit for type 1 diabetes.

A study using an insulin B-chain altered peptide ligand enrolled 188 subjects age 10–35 years (127). Subjects were randomized to one of four groups—three doses of the drug or placebo. After 2 years, there was no difference in beta cell function among the four groups.

Another study evaluated a plasmid-encoded proinsulin (128). Subjects age 18–40 years were randomized to one of five groups—four doses of the drug or placebo. Although beta cell function improved at one time point for one of the four doses, the overall intervention failed to show benefit.

A pilot safety study with a proinsulin peptide enrolled 48 subjects randomized to one of two dose groups or placebo (129). The study showed no safety issues and serves as a basis for additional studies.

Another small pilot study has been done with the combination of low-dose ATG and pegylated granulocyte colony-stimulating factor (GCSF) (130). This study enrolled 25 subjects age 12–45 years with type 1 diabetes of 4–24 months duration, randomized 2:1 to active treatment or placebo. Subjects received intravenous ATG (or placebo) over 2 days, followed by subcutaneous GCSF every 2 weeks for six doses. At the end of 1 year, beta cell function was preserved, as measured by MMTT. At the end of 2 years, the difference between groups was no longer statistically significant (131). A larger study in recent-onset Stage 3 type 1 diabetes is being conducted to pursue these observations (132).

The Diabetes and Atorvastatin (DIATOR) Trial randomized 89 subjects age 18–39 years to atorvastatin or placebo, on the basis that atorvastatin appears to have immunomodulatory properties (133). This provocative study did not meet its primary outcome (difference in C-peptide between groups at 18 months). However, when the authors examined the decline in C-peptide within the atorvastatin group, there was a nonsignificant decline, whereas the decline in C-peptide within the placebo

group was significant (133). A further analysis suggested that individuals with markers of inflammation may be the ones that benefit (134). Because atorvastatin is a common, orally administered generic drug, further evaluation of atorvastatin may be warranted.

A small pilot study evaluated etanercept, a blocker of the proinflammatory cytokine TNF (135). Although only 18 subjects were enrolled, the etanercept group had increased beta cell function at 6 months, whereas the placebo group had decreased beta cell function at that time, thus achieving statistical significance between groups.

A small safety study, involving 24 subjects, evaluated three dosing regimens of a relatively low dose of IL-2 (136). There was an increase in regulatory T lymphocytes, and no safety issues emerged, including no decline in C-peptide that was reported with higher doses of IL-2 in combination with rapamycin (125).

One study examined the effects of the combination of sitagliptin and lansoprazole in patients with recent-onset Stage 3 type 1 diabetes (137). The rationale of this study was that a dipeptidyl-peptidase 4 (DPP-4) inhibitor (sitagliptin) would increase serum levels of glucagon-like peptide-1 (GLP-1), while a proton pump inhibitor (lansoprazole) would increase serum levels of gastrin. In experimental animals, the combination GLP-1 and gastrin has been shown to increase beta cell mass and function. The human study—REPAIR T1D—randomized 68 subjects age 11–36 years in a 2:1 design to receive either the combination of sitagliptin and lansoprazole or placebo for both drugs. At 1 year, there was no difference in the rate of decline of beta cell function comparing treated subjects and control subjects (137).

In 2007, a group of investigators from Brazil reported an open label trial of 15 patients with Stage 3 type 1 diabetes age 13–31 years diagnosed within the previous 6 weeks, who were treated with the combination of high-dose immunotherapy,

with cyclophosphamide and ATG, together with nonmyeloablative autologous hematopoietic stem cell therapy (AHSCT) using CD34+ cells isolated from bone marrow (138). They reported that during 7–36 months of follow-up, 14 of 15 subjects became insulin free. Subsequently, they updated their findings in a total of 23 subjects, asserting that 20 had achieved freedom from insulin therapy, with 12 of them maintaining that for a mean of 31 months (139,140). Additional studies from Poland and China were subsequently conducted, and the Polish and Chinese data have been summarized (141). Their findings confirmed that a substantial number of subjects achieved insulin independence. However, substantial side effects occurred, including a death from *Pseudomonas* sepsis (141), and the death rate in other disease states with AHSCT can be as high as 25%. In addition, these were all nonrandomized open label studies, with incomplete characterization of the subjects, so it is not clear that all had autoimmune type 1 diabetes.

ONGOING INTERVENTION STUDIES
Thus, several provocative studies demonstrating at least transient preservation of beta cell function have been conducted, but no controlled studies have demonstrated sufficient sustained beta cell function, such that insulin therapy is not required. Additional studies are underway with a variety of approaches (132,142, 143,144,145,146,147,148,149,150,151, 152). It may be that a combination approach is needed, perhaps one that combines an anti-inflammatory agent targeting innate immunity, with an immunomodulatory agent targeting adaptive immunity, with agents that stimulate regulatory immunity, and an agent that helps preserve beta cell health (153).

LIST OF ABBREVIATIONS

A1c . . . . glycosylated hemoglobin
AAT . . . . alpha-1 antitrypsin
AHSCT . . . . autologous hematopoietic stem cell therapy
ATG . . . . antithymocyte globulin
BCG . . . . bacille Calmette-Guerin
CI . . . . confidence interval
DENIS . . . . German (Deutsch) Nicotinamide Diabetes Intervention Study
DHA . . . . docosahexaenoic acid
DIPP . . . . Diabetes Prediction and Prevention Study
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
GAD . . . . glutamic acid decarboxylase
GCSF . . . . granulocyte colony-stimulating factor
GLP . . . . glucagon-like peptide
HLA . . . . human leukocyte antigen
HR . . . . hazard ratio
IA2 . . . . islet antibody-2
IAA . . . . insulin autoantibodies
ICA . . . . islet cell antibodies
IL . . . . interleukin
INIT . . . . Intranasal Insulin Trial
ITN . . . . Immune Tolerance Network
MMTT . . . . mixed meal tolerance test
NIP . . . . Nutritional Intervention to Prevent Type 1 Diabetes
OR . . . . odds ratio
Pre-POINT . . . . Primary Oral Insulin Therapy Study
TNF . . . . tumor necrosis factor
TRIGR . . . . Trial to Reduce the Incidence of Diabetes in the Genetically at Risk

CONVERSIONS

Conversions for A1c and glucose values are provided in *Diabetes in America Appendix 1 Conversions*.

DUALITY OF INTEREST

Dr. Skyler received personal fees from Dexcom, Debiopharm, Genentech, ImmunoMolecular Therapeutics, Intarcia, Intrexon, INNODIA, Eli Lilly and Company, Merck, Novo Nordisk, Orgenesis, and Sanofi Genzyme outside the submitted work. Drs. Krischer, Becker, and Rewers reported no conflicts of interest.

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