Prevention and Reversal of Type 1 Diabetes—Past Challenges and Future Opportunities

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Over the past three decades there have been a number of clinical trials directed at interdicting the type 1 diabetes (T1D) disease process in an attempt to prevent the development of the disease in those at increased risk or to stabilize—potentially even reverse—the disease in people with T1D, usually of recent onset. Unfortunately, to date there has been no prevention trial that has resulted in delay or prevention of T1D. And, trials in people with T1D have had mixed results with some showing promise with at least transient improvement in β-cell function compared with randomized control groups, while others have failed to slow the decline in β-cell function when compared with placebo. This Perspective will assess the past and present challenges in this effort and provide an outline for potential future opportunities.

THE BEGINNINGS

The first randomized, double-masked, controlled trials with sufficient statistical power to give confidence for the outcome were conducted in the mid-1980s with cyclosporine (1,2). Two large studies were conducted—the French cyclosporine study that included 122 patients with type 1 diabetes (T1D) aged 15–40 years who had been symptomatic for 6 months or less and were on insulin therapy for 2 months or less (1) and the Canadian-European cyclosporine study that included 188 subjects aged 9–35 years who had been symptomatic for 14 weeks or less and were on insulin therapy for 6 weeks or less (2). Both trials used as their primary outcome the achievement of remission defined two ways. First, "complete remission" was defined as good metabolic control (fasting glucose $<$140 mg/dL [7.8 mmol/L], postprandial glucose $<$200 mg/dL [11.1 mmol/L], HbA_{1c} $\leq$7.5%) in the absence of insulin treatment. Second, "partial remission" was defined as good metabolic control with insulin dose $<$0.26 units/kg per day. Both studies found a greater proportion of cyclosporine patients than placebo patients achieving and maintaining remissions. In both studies, cyclosporine could be given for 1 year, with stopping rules in place that led to blinded substitution of placebo for cyclosporine if remission was lost. Although both studies found that cyclosporine had superior efficacy than placebo, the magnitude and duration of benefit did not appear sufficient to justify cyclosporine treatment in clinical practice, given the potential of cyclosporine-induced nephrotoxicity. The importance of the studies, however, was that they demonstrated the impact of immune intervention on the evolution of T1D, in a sense fulfilling Koch postulates, which were developed for infectious diseases, but the response to immune therapy can be considered as indicative that T1D is immune mediated.

CHALLENGES COMPLICATING PREVIOUS STUDIES

Since the completion of the early trials, particularly during the past decade, a number of additional randomized, double-masked, adequately powered, controlled

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clinical trials have been conducted using many different immunological strategies. For the most part, these have been disappointing, with none showing unambiguous benefit in preserving β-cell function. Some studies have shown transient benefit with anti-CD3 monoclonal antibodies targeting T cells (Fig. 1*A* and *B*) (3–6), an anti-CD20 monoclonal antibody (i.e., rituximab) targeting B cells (Fig. 1*C*) (7,8), and with a costimulation blocking agent (i.e., abatacept) that prevents immune activation (Fig. 1*D*) (9,10). One pilot study combining antithymocyte globulin (ATG) and granulocyte colony-stimulating factor (GCSF) suggested benefit (Fig. 1*E*) but awaits confirmation in a larger trial (11).

A number of other strategies have been without benefit in preserving β-cell function (12–17). In addition, some studies had ambiguous effects, not meeting the primary outcome measure (i.e., preservation of β-cell function) but showing potential benefit either on secondary outcome measures or mechanistic measures or in only a subgroup of subjects (18–20).

Thus, most immune intervention trials in T1D have either failed to achieve success in preserving β-cell function or have met that hurdle but have nonetheless shown only a transient effect. This has resulted in a flurry of editorial and commentary articles in peer-reviewed journals that take a bleak look at the field. Rather than looking negatively on the results to date, an opportunity exists to examine the details of previous studies to identify what can be learned and what can be applied to future studies. To that end, several problems are notable, and these are detailed below.

MISLEADING PILOT STUDIES

The Diabetes Prevention Trial–Type 1 Parenteral Insulin Trial was a fully powered, randomized, controlled clinical trial that enrolled 339 relatives of patients with T1D who were estimated to have at least a 50% risk of developing T1D in the next 5 years (21). To enroll these

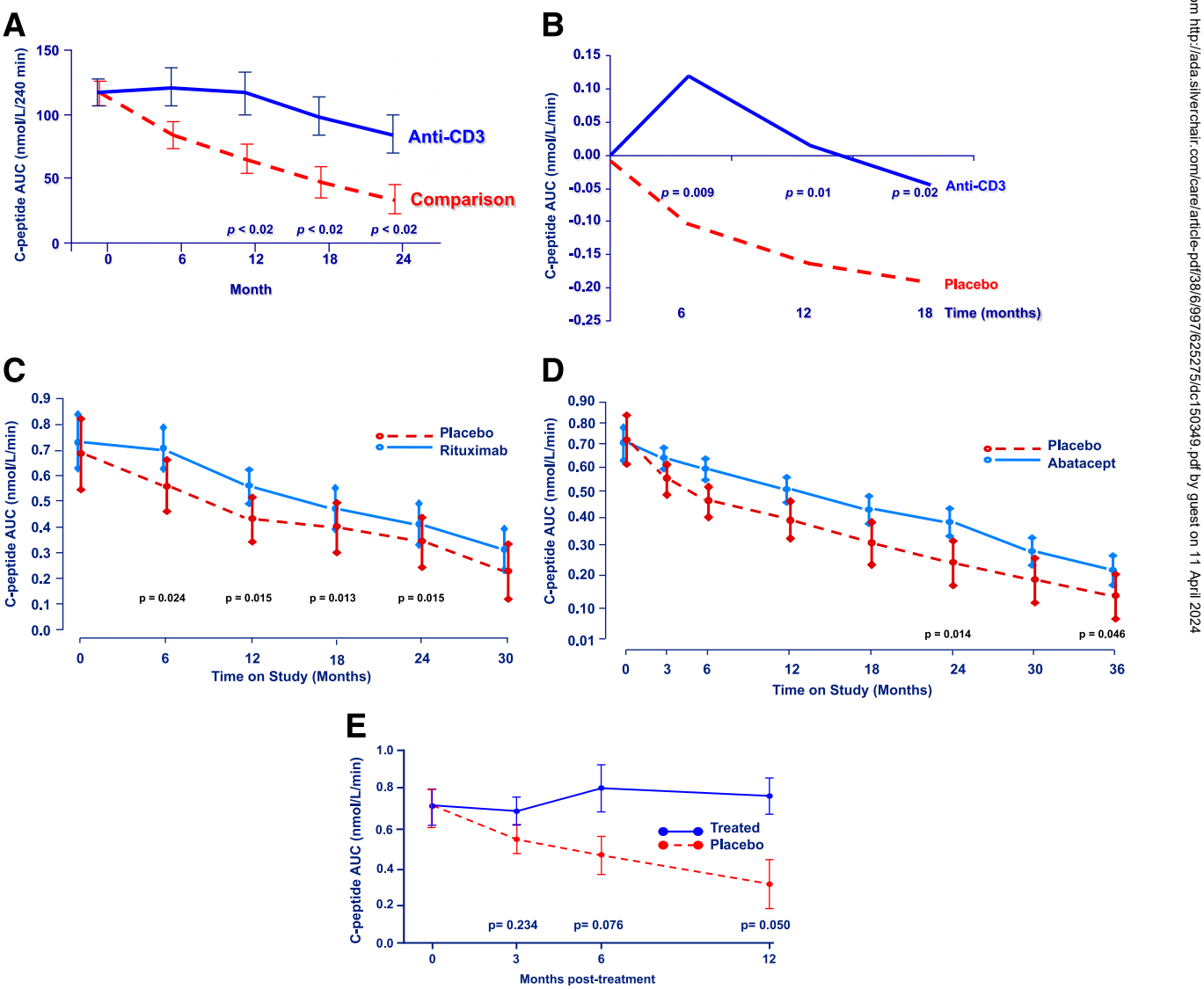

Figure 1—There has been a progressive decline in β-cell function, as measured by C-peptide, even in most studies that have been "successful," thus showing only a transient benefit, as depicted for the anti-CD3 monoclonal antibody teplizumab (*A*) (3,4), the anti-CD3 monoclonal antibody otelixizumab (*B*) (5,6), the anti-CD20 monoclonal antibody rituximab (*C*) (7,8), and the costimulation blocker abatacept (*D*) (9,10). *E*: Also shown is the preservation of β-cell function in a pilot study (only 25 subjects randomized) with the combination of low-dose ATG plus GCSF (11), in which there is preservation of β-cell function at 1 year, something needing confirmation in a larger study.

subjects, more than 93,000 relatives were screened. The trial showed no difference in the rate of development of T1D (Fig. 2*A*) (21). This was a surprising outcome, as two pilot studies had suggested that the intervention—low-dose insulin—could delay the disease. One of those apparently promising pilot studies was a nonrandomized study of 12 individuals offered the intervention, 7 of whom declined and were analyzed as a comparison group, along with a group of historical control subjects of undefined number (Fig. 2*B*) (22). Life-table analysis suggested a statistically significant (P = 0.002) and dramatic difference in rate of development of T1D. Interestingly, the treated group was younger (mean age 10 years) than the untreated group (mean age 20.7 years), and one would have expected younger individuals to progress faster, which did not happen. In the other pilot trial, 14 subjects were randomized to insulin treatment or to the control group, with the insulin group having longer diabetes-free survival ($P < 0.03$) (23).

The first study in T1D using GAD in a vaccine formulation with alum (GAD-alum) randomized 70 subjects, aged 10–18 years, within 18 months of diagnosis, to receive either two doses of GAD-alum or two doses of alum alone (24). The primary outcome measure, fasting C-peptide at 15 months, showed no difference between groups. However, mixed-meal tolerance test (MMTT)-stimulated C-peptide was higher in GAD subjects who were treated within 6 months of diagnosis, but this included only 11 subjects treated with GAD and 14 treated with placebo (Fig. 2*C*). On the basis of this secondary subgroup outcome, three additional studies—all enrolling subjects within 3 months of diagnosis—were undertaken, including two phase 3 trials. A TrialNet study enrolled 145 subjects aged 3–45 years (Fig. 2*D*), a European phase 3 trial enrolled 334 subjects aged 10–20 years (Fig. 2*E*), and a U.S. phase 3 trial enrolled

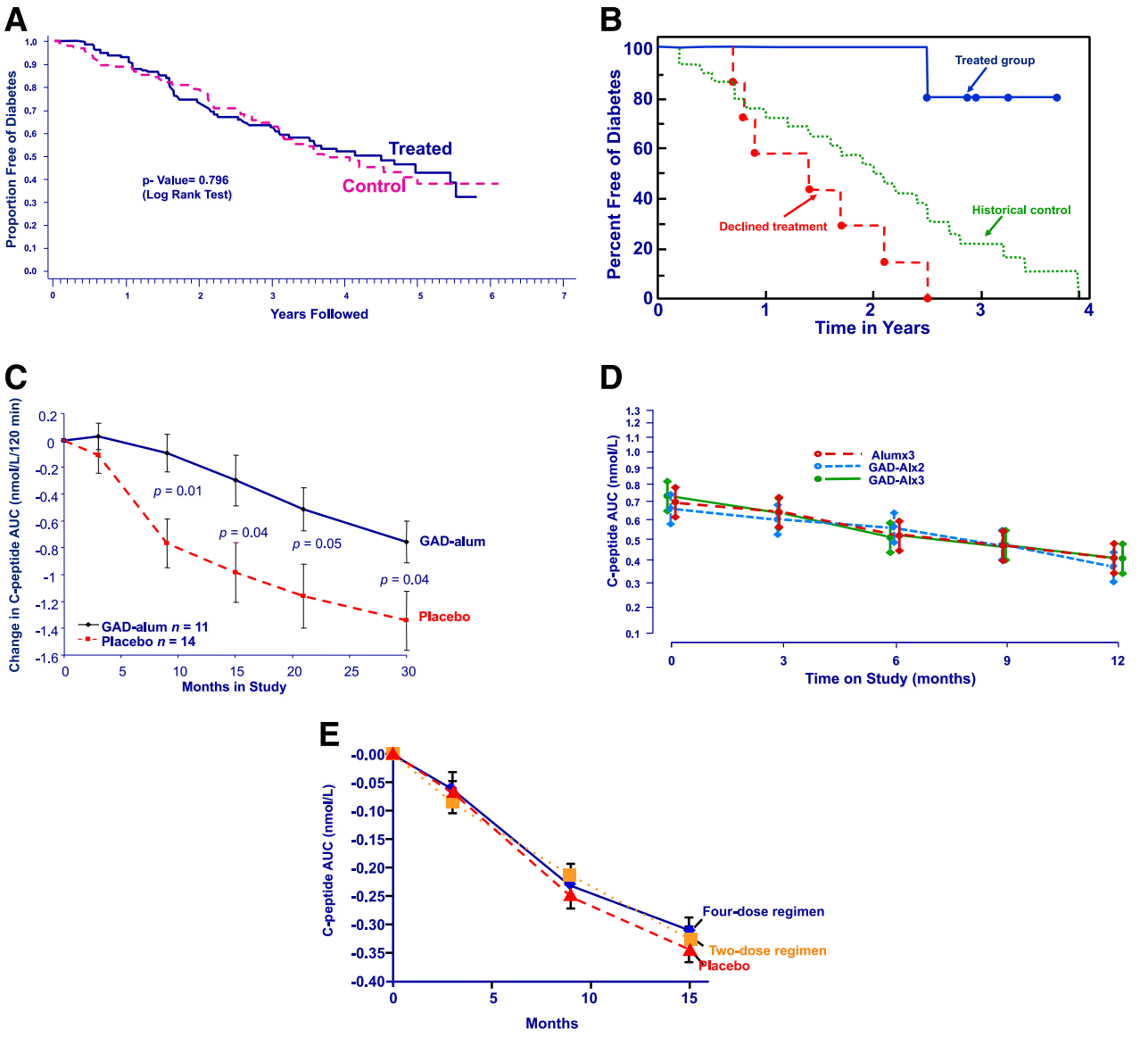

Figure 2—*A*: Life-table analysis showing lack of benefit in the fully powered DPT-1 Parenteral Insulin Trial (21), despite perceived benefit (by life-table analysis) in a small pilot study (*B*) (22). *C*: Putative benefit in a small subgroup (those treated within 6 months of diagnosis) in a GAD-alum vaccine trial (24) that was not confirmed in two larger trials (*D* and *E*) with GAD-vaccine (12,13).

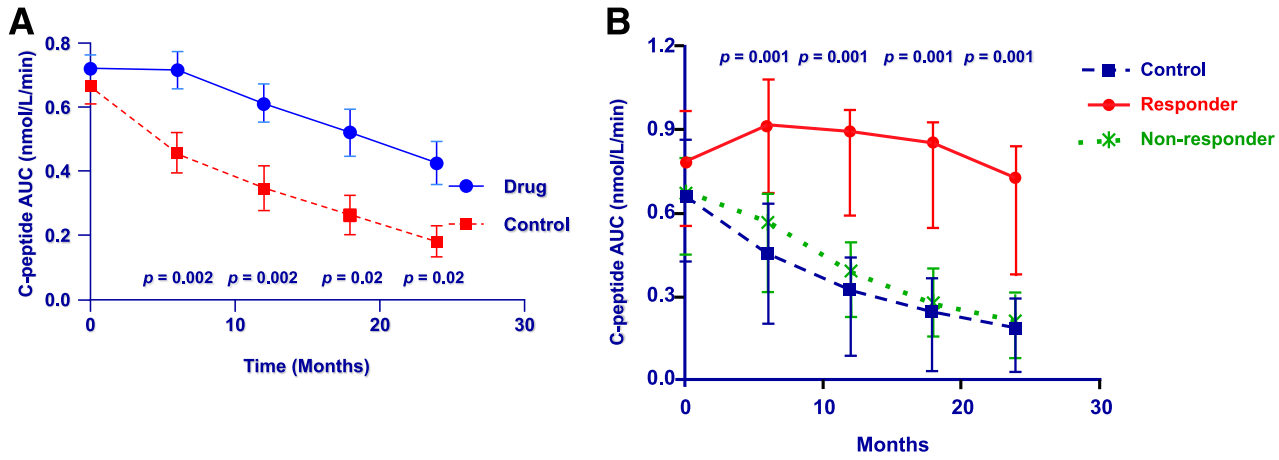

Figure 3—*A*: Transient effect of the anti-CD3 monoclonal antibody teplizumab in all treated subjects in one study (62). *B*: In that same study, retention of β-cell function for 2 years in responders, whereas nonresponders were identical to the comparison control group.

328 subjects aged 10–20 years (12,13,25). In all three of these trials, there was no evidence of a treatment effect with GAD-alum. Yet, it should be appreciated that the success of GAD in mice was approached differently. For example, the GAD-alum studies perhaps used the wrong formulation (with adjuvant). The studies could have used GAD-alum by the wrong route (subcutaneous) and at the wrong time (after clinical diagnosis of T1D). Although GAD vaccine still may be able to prevent or delay the development of T1D if used as a vaccine prior to disease onset, the results of these trials have greatly diminished the enthusiasm for GAD that once existed in our field.

Another approach considered was DiaPep277. DiaPep277 is a peptide derived from positions 437–460 of the human heat shock protein 60 (Hsp60), named peptide 277, that is alleged to induce anti-inflammatory T cells. The first report of its use in T1D was a pilot study in which 35 subjects, aged 16–55 years, with recent onset T1D, received either three doses of DiaPep277 or three doses of placebo (26). Primary outcome was glucagon-stimulated C-peptide at 10 months, which was higher in the DiaPep277 group than in the placebo group, an effect that was sustained with follow-up to 18 months (27). Four additional phase 2 studies showed results that were ambiguous at best, with no clear benefit (28–30). Nonetheless, the sponsor mounted two full-scale phase 3 trials. The first enrolled 457 subjects, aged 16–45 years, with recent-onset T1D, who received injections of DiaPep277 or placebo quarterly for 2 years (14,15). The initial report alleged that glucagon-stimulated test (GST) of C-peptide at 24 months was improved in the DiaPep277 group versus the placebo group, although there was no difference in MMTT-stimulated C-peptide. However, MMTT was the original primary outcome measure and therefore was measured at randomization (month 0) and after 6, 12, 18, and 24 months—a total of 5 measurements. As GST originally was a secondary outcome measure, it was performed at month 1 (defined as "baseline" for the GST but 1 month after the first treatment had been given) and at 12 and 24 months—a total of 3 measurements. The authors intended, initially, to have MMTT be the primary outcome measure—they performed the first MMTT before initiating treatment (a true baseline measurement) and conducted the test at more frequent intervals. However, the primary outcome measure was changed from the MMTT to the GST. Specifically, it was stated "the study protocol was amended and the Statistical Analysis Plan was planned and finalized before the study was unblinded, with the GST clearly defined as the primary endpoint" (14). Had that really been the case, it raised questions of why differences in these two outcome measures existed and led to the question of whether both measures would be needed in future trials (31). However, subsequently the article was retracted with a statement that there had been evidence uncovered that some employees of the sponsor had engaged in serious misconduct, including collusion with a third-party biostatistics firm to improperly receive unblinded trial data and to use such data in order to manipulate the analyses to obtain a favorable result.

Another pilot study involved the use of Bacillus Calmette-Guérin (BCG) vaccine in six subjects with long-standing T1D who were randomized to receive either two doses of BCG or placebo (32). The authors claimed that the BCG subjects, and one of the three control subjects who developed acute Epstein-Barr virus (EBV) infection, showed increases in dead insulin-autoreactive T cells and induction of regulatory T cells. They also claimed that there was transient increase in C-peptide levels not seen in a nonrandomized cohort of subjects with T1D in their institution. They have garnered a lot of publicity for this very tiny study. Although the authors reported an increase in C-peptide levels using an ultrasensitive assay, there are no data that the trivial increases in C-peptide have any biological importance. Moreover, two larger previous randomized clinical trials of BCG, involving 26 and 47 subjects, showed no effect of BCG on preservation of β-cell function, but in both trials there was a trend to greater decline of β-cell function in the BCG group than in the control group (33,34).

What can one then conclude about the value of pilot studies in this area? Essentially, the bottom line is that pilot studies must be viewed with great caution and definitive answers can only be obtained by adequately powered randomized controlled trials.

FAILURE TO DISTINGUISH TRANSIENT SYMPTOMS FROM SERIOUS ADVERSE EVENTS

A number of interventions used in recent-onset T1D have shown transient symptoms related to cytokine release

Table 1—Immune intervention trials in T1D

Study name	Intervention	Outcome	Result	Year reported	Reference
Primary prevention studies					
Finnish TRIGR pilot	Casein hydrolysate formula	Autoantibodies	Apparent benefit	2010	66
TRIGR	Casein hydrolysate formula	Autoantibodies	No difference	2014	67
FINDIA	Insulin-free whey-based formula	Autoantibodies	Apparent benefit	2012	68
BABYDIET	Gluten-free diet	Autoantibodies	No difference	2011	69
TRIGR	Casein hydrolysate formula	Diagnosis of T1D	*	Ongoing	67
Secondary prevention studies					
DENIS	Nicotinamide	Diagnosis of T1D	No difference	1998	70
ENDIT	Nicotinamide	Diagnosis of T1D	No difference	2004	71
DPT-1 Parenteral Insulin	Injected insulin	Diagnosis of T1D	No difference	2002	21
DPT-1 Oral Insulin	Oral insulin	Diagnosis of T1D	No difference	2005	20
Belgian parenteral insulin	Injected insulin	Diagnosis of T1D	No difference	2009	51
DIPP birth cohort	Nasal insulin	Diagnosis of T1D	No difference	2008	52
DIPP sibling cohort	Nasal insulin	Diagnosis of T1D	No difference	2008	52
INIT II	Nasal insulin	Diagnosis of T1D	*	Ongoing	60
DIAPREV-IT	GAD	Diagnosis of T1D	*	Ongoing	58
TrialNet oral insulin	Oral insulin	Diagnosis of T1D	*	Ongoing	59
TrialNet teplizumab	Anti-CD3, teplizumab	Diagnosis of T1D	*	Ongoing	72
TrialNet abatacept	Abatacept	Diagnosis of T1D	*	Ongoing	73
Studies in recent-onset T1D					
French cyclosporine	Cyclosporine	Remission	Benefit	1986	1
Canadian-European cyclosporine	Cyclosporine	C-pep $\geq$0.6 nmol/L or noninsulin treated	Benefit	1988	2
Azathioprine + glucocorticoids	Azathioprine and prednisone	Peak C-pep/glucose ratio	Benefit	1988	74
Azathioprine, adults	Azathioprine	Remission	Benefit	1985	75
Azathioprine, children	Azathioprine	Partial remission	No difference	1989	76
Linomide French trial	Linomide	Glucagon-stimulated C-pep	Benefit	1998	77
BCG	BCG vaccine	Glucagon-stimulated C-pep	No difference	1998	33
BCG	BCG vaccine	Primary, remission; secondary, MMTT C-pep	No difference	1999	34
French oral insulin	Oral insulin	Glucagon-stimulated C-pep	No difference	2000	53
Italian oral insulin	Oral insulin	Fasting C-pep	No difference	2000	54
U.S. oral insulin	Oral insulin	Loss of C-pep	#	2004	55
Herold anti-CD3	Teplizumab	MMTT C-pep	Benefit	2002, 2005	3,4
Keymeulen anti-CD3	Otelixizumab	C-pep after clamp	Benefit	2005, 2010	5,6
Protégé	Teplizumab	Insulin $<$0.5 unit/kg + HbA_{1c} $<$6.5%	No difference	2011, 2013	35,36
Protégé Encore	Teplizumab	Insulin $<$0.5 unit/kg + HbA_{1c} $<$6.5%	*	2011	78
DEFEND-1	Otelixizumab	MMTT C-pep	No difference	2014	37
DEFEND-2	Otelixizumab	MMTT C-pep	No difference	2014	38
AbATE (ITN study)	Teplizumab	MMTT C-pep	Benefit	2013	62
DELAY	Teplizumab	MMTT C-pep	Benefit	2013	79
GAD pilot	GAD-alum vaccine	Fasting C-pep	Apparent benefit in secondary outcome in subgroup	2008	24
GAD TrialNet	GAD-alum vaccine	MMTT C-pep	No difference	2011	12
GAD Europe	GAD-alum vaccine	MMTT C-pep	No difference	2012	13
GAD U.S. (DiaPrevent)	GAD-alum vaccine	MMTT C-pep	N/A	2011	25
DiaPep–Israeli adults	DiaPep277 peptide	Glucagon-stimulated C-pep	Benefit	2001, 2007	26,27
DiaPep–Israeli pediatrics	DiaPep277 peptide	MMTT C-pep	No difference	2007	28

Continued on p. 1002

Table 1—Continued

Study name	Intervention	Outcome	Result	Year reported	Reference
DiaPep–Belgian adults	DiaPep277 peptide	Glucagon-stimulated C-pep	Benefit at one dose	2007	29
DiaPep–Europe adults	DiaPep277 peptide	Glucagon-stimulated C-pep	No difference	2007	30
DiaPep–Europe pediatrics	DiaPep277 peptide	Glucagon-stimulated C-pep	No difference	2007	30
DiaPep–phase III	DiaPep277 peptide	Glucagon-stimulated C-pep	ǂ	2014	14,15
MMF/DZB	Mycophenolate mofetil with/without daclizumab	MMTT C-pep	No difference	2010	16
Anti-CD20 TrialNet	Anti-CD20 rituximab	MMTT C-pep	Benefit	2009, 2014	7,8
Abatacept TrialNet	Abatacept	MMTT C-pep	Benefit	2011, 2014	9,10
Canakinumab TrialNet	Anti-IL1β canakinumab	MMTT C-pep	No difference	2013	17
START thymoglobulin ITN	Thymoglobulin	MMTT C-pep	No difference	2013	18
T1DAL–alefacept ITN	Alefacept	MMTT C-pep	No difference	2013	19
IL-2 & rapamycin safety ITN	IL-2 and rapamycin	MMTT C-pep	Transient worsening	2012	45
AIDA anakinra trial	Anakinra	MMTT C-pep	No difference	2013	17
α1-Antitrypsin	α1-Antitrypsin	MMTT C-pep	§	2014	80
Altered peptide ligand	B9–23 altered peptide ligand	MMTT C-pep	No difference	2009	56
Plasmid-encoded proinsulin	Plasmid-encoded proinsulin	Safety MMTT C-pep	¶	2013	57
Proinsulin peptide	Proinsulin peptide	Safety study	No safety issues	2009	61
ATG–GCSF trial	ATG and GCSF	MMTT C-pep	Benefit	2015	11
DIATOR	Atorvastatin	MMTT C-pep	No difference	2011	81
Etanercept	Etanercept	MMTT C-pep	Benefit	2009	82
Low-dose IL-2 safety trial	IL-2 (3 doses)	T-reg number	Increased	2013	46
REPAIR-T1D	Sitagliptin and lansoprazole	MMTT C-pep	No difference	2014	48
AHSCT + profound immunosuppression	Cyclophosphamide, GCSF, ATG, AHSCT	MMTT C-pep	Benefit	2007, 2009, 2009	83–85
AHSCT + profound immunosuppression	Cyclophosphamide, GCSF, ATG, AHSCT	MMTT C-pep	Benefit	2014	86
ATG–GCSF trial	ATG and GCSF	MMTT C-pep	*	Ongoing	87
EXTEND trial	Tocilizumab	MMTT C-pep	*	Ongoing	88
Otelixizumab dose-ranging trial	Otelixizumab	MMTT C-pep	*	Ongoing	89
α1-Antitrypsin trial	α1-Antitrypsin	Basal C-pep	*	Ongoing	90
α1-Antitrypsin trial	α1-Antitrypsin	MMTT C-pep	*	Ongoing	91
Ustekinumab pilot	Ustekinumab	Safety	*	Ongoing	92
Imatinib trial	Imatinib	MMTT C-pep	*	Ongoing	93
Tauroursodeoxycholic acid trial	Tauroursodeoxycholic acid	MMTT C-pep	*	Ongoing	94
DIABGAD	GAD-alum & vitamin D with/without ibuprofen	MMTT C-pep	*	Ongoing	95
Proinsulin peptide	Proinsulin peptide	Safety	*	Ongoing	96
Methyldopa	Methyldopa	Inhibition of DQ8 Ag	*	Ongoing	97
Low-dose IL-2	IL-2	T-reg number	*	Ongoing	98

AHSCT, autologous hematopoietic stem cell transplantation; C-pep, C-peptide; INT, Immune Tolerance Network; T-reg, regulatory T cell. *Data not yet available. #Data ambiguous—authors claim benefit but only seen in one dose in post hoc subgroup. N/A—actual data not available; press release announced negative result and study discontinuation. ǂArticle retracted. §Data ambiguous—authors claim benefit but single-arm trial and "benefit" unclear. ¶Data ambiguous (as discussed in text).

syndrome, including headache, fever, and hypotension, at the time of infusion of the treatment. These symptoms are not intolerable and are both transient and fully reversible. Thus, they do not constitute major adverse effects that would justify withdrawal of the subject from the study. Cytokine release syndrome has been seen with infusions of anti-CD3 monoclonal antibodies (3,5), rituximab (7), and thymoglobulin (11,18). Yet, although the infusions have been completed within the first days or within the first month after randomization, beneficial effects from these interventions have been seen 12 months to 4 years after randomization (3–7,11). Thus, subjects may have some discomfort, but there needs to be a reality check as to the potential benefit versus the discomfort. A few days of symptoms early on need to be balanced with a sustained beneficial outcome over a protracted time frame.

Some of these trials have used antihistamine and analgesic prophylaxis to obviate symptoms related to cytokine release. At least one trial also used low doses of glucocorticoids (18) but only in the experimental group, not in

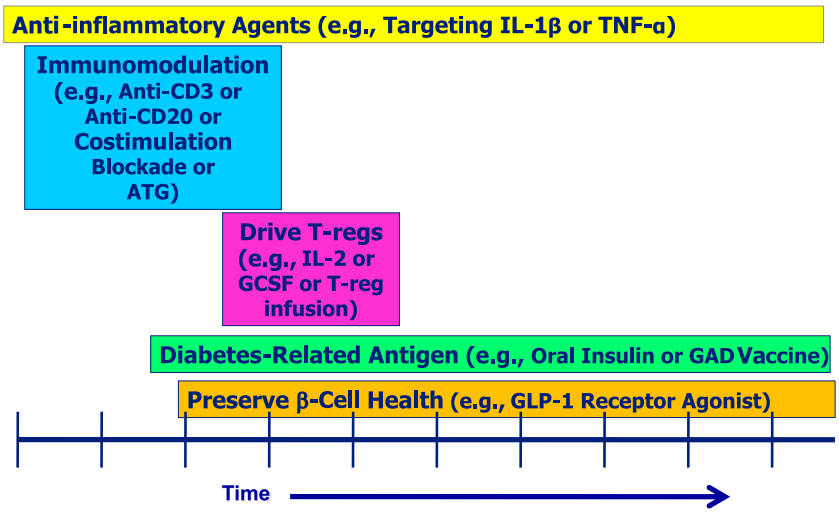

Figure 4—Potential scheme of combination therapy using agents with complementary effects. This scheme includes anti-inflammatory therapy targeting innate immunity, immunomodulatory therapy targeting adaptive immunity, therapy driving regulatory immunity, antigen-based therapy directing regulation to β-cells, and an agent promoting β-cell health. T-reg, regulatory T cell.

the placebo group. One might consider giving the same prophylaxis to both experimental and placebo groups in order to minimize the risk of unblinding.

ERRORS IN TRIAL DESIGN

There have been extensive studies with two anti-CD3 monoclonal antibodies—teplizumab and otelixizumab. The first study with teplizumab (given over 14 days) demonstrated a slower decline of β-cell function (by MMTT) at 1 year (3), with sustained improvement on β-cell function at 2 years (4). Meanwhile, the first study with otelixizumab (given over 6 days) showed a slower decline of β-cell function (measured using a hyperglycemic clamp followed by glucagon stimulation) at 18 months (5). After 4 years of follow-up, although β-cell function was not measured, the otelixizumab group had lower insulin requirements despite similar glycemic control as measured by HbA_{1c} (6). Thus, effects of a 6-day treatment course appeared to be evident 4 years later.

The results from these early studies with anti-CD3 led to the initiation of phase 3 clinical trials with both agents. Unfortunately, the phase 3 studies did not meet their primary outcome criteria. For teplizumab, the primary outcome was the combination of HbA_{1c} <6.5% and insulin dose <0.5 units/kg/day (35). This outcome measure was arbitrarily selected without sufficient data to justify its selection. Moreover, by using a composite outcome that requires a subject to meet two criteria, the outcome becomes a dichotomous measure that dilutes the effect of two continuous variables—HbA_{1c} and insulin dose. More important, when the conventional outcome measure of C-peptide was assessed, there was evidence of efficacy both at 1 year (35) and at 2 years (36) 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 U.S., 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 (35). In addition, the phase 3 study also enrolled subjects in South Asia. Although these subjects met the clinical criteria used for enrollment, it is important to note that typical immune-mediated T1D (also called type 1A diabetes) is a disease principally of Europoid Caucasians. Enrollment of Asian subjects may have confounded the results. Another issue was that the control group in the phase 3 study maintained residual C-peptide to a greater extent and longer than expected, which was especially true for adult subjects. This made it more difficult to detect differences between groups.

For otelixizumab, the phase 3 studies used a dose that was one-sixteenth (total of 3.1 mg over 8 days) of that used in the original phase 2 study (total of 48 mg), in an effort to avoid any side effects (37,38). One has to question what the investigators were trying to avoid. The one noninfusion-related side effect seen in the first trial with otelixizumab was transient EBV reactivation (39). Although the authors concluded that such EBV reactivation was of no apparent clinical concern over the long term, others have asserted that this must be avoided at all costs (40). I was Chair of the Data Safety Monitoring Committee for that study, and prior to the study the committee had concluded that transient EBV reactivation was possible and would neither constitute a reason to halt the study nor was a side effect that needed to be avoided. So, in the phase 3 trials with use of the lower dose, side effects were obviated; however, beneficial effects were also completely obviated. This unfortunate dose reduction reminds us that all effective therapies are likely to have some side effects and that if one lowers the dose to eliminate all side effects, the drug may no longer have benefit.

DOSING ISSUES

Getting the dose right is important. In addition to the otelixizumab dosing issue, several other examples are worth noting. Interleukin-2 (IL-2) stimulates regulatory T cells at low doses but also stimulates effector T cells at higher doses (41). Thus, it has been noted that the use of IL-2 is a double-edged sword (42). In other conditions (e.g., hepatitis-C virus–induced vasculitis and graft-versus-host disease), low-dose IL-2 has shown beneficial effects (43,44). The first human study of IL-2 in T1D used a relatively high dose and combined its use with rapamycin (45). An adverse effect on β-cell function was observed. A subsequent study using low-dose IL-2 appeared to be relatively safe, without a decrease in β-cell function and with the expected increase in regulatory T cells (46). Therefore, studies are currently under way to study low-dose IL-2 in T1D.

Another example of a dosing issue is thymoglobulin (ATG). In a study using a relatively high dose of ATG, no beneficial effect was seen, and it was observed that there was suppression of both effector and regulatory T cells (18). In another study in which a lower dose of ATG was used, regulatory T cells were not suppressed (11). Interpretation of that study is confounded, however, because low-dose ATG was used in combination with GCSF.

A related issue providing an additional confounder is choosing surrogate agents. A study in NOD mice found that combination therapy with glucagon-like peptide 1 (GLP-1) and gastrin restores normoglycemia (47). This combination resulted in increases in pancreatic insulin content, β-cell mass, β-cell proliferation, and β-cell neogenesis; a reduction in β-cell apoptosis; and a beneficial effect on the immune response (47). It seemed like a natural combination to test in human beings. Yet, rather than testing the combination of GLP-1 and gastrin, a study evaluated the combination of the dipeptidyl peptidase-4 inhibitor sitagliptin and the proton-pump inhibitor lansoprazole, as these agents, respectively, increase circulating levels of GLP-1 and gastrin (48). The primary end point was not achieved, but not all participants had the expected increases in GLP-1 and gastrin levels. Thus, they effectively did not get the doses that may have been needed to achieve the desired effects.

ANTIGEN-BASED THERAPIES HAVE NOT WORKED YET

The desirability of antigen-based immunotherapy is grounded on the notion that such therapies are specific for T1D and are unlikely to have adverse off-target effects. Thus, they should have a high degree of safety. The two diabetes-specific antigens that have been used are insulin and GAD, both of which had great success in animal models of T1D (49,50). Yet, to date, there has been no unambiguous success with antigen-based therapies in human T1D. GAD-alum vaccine has failed in new-onset T1D (12,13). Injected insulin has failed in prevention trials (21,51). Nasal insulin has failed in prevention trials (52). Oral insulin has failed in new-onset T1D (53–55) and did not meet its primary outcome in a prevention trial (20). An altered peptide ligand of insulin failed in new-onset T1D (56). A plasmid-encoding proinsulin was claimed to have a benefit on β-cell function, but actually the effect was seen in but one of four doses tested and only at one time point (57). Moreover, the number of subjects studied at each dose was small, and there did not appear to be statistical adjustment for multiple comparisons. The field is eagerly awaiting the conclusion of ongoing GAD-alum (58), oral insulin (59), and nasal insulin (60) prevention trials and of the development of an approach that uses a combination of peptides derived from proinsulin (61). Yet, the desirable concept of an antigen-based therapy sustains the efforts to find an effective one, perhaps as a component of a combination therapeutic approach.

NOT ALL PEOPLE MAY RESPOND TO THERAPY

In one study using the anti-CD3 monoclonal antibody teplizumab, a group of "responders" to treatment was identified, who at 2 years maintained C-peptide better than the randomized but untreated comparison group (Fig. 3) (62). In that study, responders constituted 48% of subjects treated with teplizumab. Interestingly, the responders not only did better than the comparison group but also, as a group, actually maintained β-cell function essentially at the level seen at randomization, whereas the nonresponders had lost β-cell function at a rate similar to the comparison group. Had the analysis been confined to the total treated group—including both responders and nonresponders—the full retention of β-cell function in nearly half of the subjects would have been missed. Some type of responder analysis should be applied to all intervention studies. Indeed, it has been suggested that a responder analysis be included in the statistical plan for all T1D intervention studies, and for that purpose, the definition of a responder should be the maintenance of 100% of baseline β-cell function (63).

A fundamental question is why some subjects fail to respond. It could be that the immunotherapy used was ineffective (at least at the dose tested), that the immunological process—perhaps a relapsing and remitting one—was in a latent period at the time of drug administration and thus not responsive to immunotherapy, that β-cell mass or β-cell function had already deteriorated to a point of no return, that the immunological processes damaging β-cells are different among individuals, or for some other reason. It is important to assess potential biomarkers that might discriminate responders from nonresponders and thus might be used as enrollment criteria for future use of a given therapy.

FUTURE DIRECTIONS

As discussed above, there have been many attempts at immune intervention in T1D, with mixed results. Table 1 provides a listing of all of the major studies to date, including randomized controlled trials and other studies mentioned in this Perspective; not included are small pilot studies not discussed in this article.

It should be appreciated that in the evolution of T1D several immune pathways are involved. This complicates the design of an ideal therapeutic strategy to control the immune system and prevent the loss of β-cell function and β-cell mass. Indeed, if one pathway is controlled, another pathway may become more active. Thus, success may require that a combination approach be used. Such a combination might include (Fig. 4) one or more anti-inflammatory agents targeting innate immunity, such as agents that target IL-1 (IL-1β) or tumor necrosis factor (TNF-α); one or more immunomodulatory agents targeting adaptive immunity, such as anti-CD3, anti-CD20, or costimulation blockade (three agents that already have shown some beneficial effect on preserving β-cell function) or ATG; perhaps some agent that would drive regulatory T cells, such as low-dose IL-2 or GCSF or the infusion of regulatory T cells themselves; any of these perhaps coupled with a diabetes-related antigen that might help target the regulatory T cells to β-cells; and agents that help preserve β-cell health, such as GLP-1 (64). In such a strategy, the combination of agents is selected for having potential complementary effects. It may also be necessary to tailor the selection of agents for different individuals, i.e., we may need to move to a personalized medicine approach with different treatments for different subtypes, if these become better defined (65).

Therefore as outlined, there are many potential interventions that hold promise, particularly if they are used as components of combination therapy. Several new strategies are approaching clinical evaluation. To be successful, we must be patient, yet proceed with diligence. Moreover, it is important that trials be carefully designed, well controlled, and

have adequate sample size to assure valid interpretation.

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