The idea of treating type 1 diabetes mellitus (T1D) with immunosuppressive agents began with the recognition of this form of diabetes as a chronic autoimmune process [1]. Understanding of this process has been evolving, and with it, strategies for immune therapy. Unlike treatment with insulin, immune interventions are believed to affect the cause of the disease, but because newer data suggest a role for insulin in mediating β-cell survival and regeneration, metabolic treatment and immune intervention may need to be considered jointly. This article summarizes the evolution of current approaches to treatment and current controversies that attend these studies.

Natural history of type 1 diabetes mellitus

The autoimmune response that causes T1D begins years before clinical onset of disease, continues for years after diagnosis, and may recur years later if patients are rechallenged with an islet transplant. Initial pathologic studies of islets from patients with new-onset T1D suggested loss of approximately 90% of insulin-positive cells [2]. Early studies by Faber and colleagues [3] showed that soon after diagnosis, the production of C-peptide in patients with diabetes consuming a standard diet was about one third of that seen in normal individuals. These studies measured 24-hour C-peptide levels, which provide only an estimate of insulin production. The interpretation that insulin secretion was impaired markedly at the time of diagnosis with little evidence of recovery of β-cell mass was prevalent in the late 1980s and early 1990s. The potential benefits of immunosuppressive
therapies were not believed to justify the risks involved with drug administration.

A more recent, cross-sectional analysis of data from the Diabetes Care and Complication Trial (DCCT) showed more substantial insulin production in patients with T1D than had been previously appreciated [4]. In the DCCT, a stimulated C-peptide level of greater than 0.2 pmol/mL was associated with improved metabolic control compared with β-cell secretory levels below that level. Nearly one half of adults with T1D of 1 to 5 years’ duration had C-peptide levels greater than 0.2 pmol/mL, and 10% of subjects 5 to 15 years after diagnosis retained C-peptide of that level [4]. More recent data from our studies have shown that 100% of subjects have stimulated C-peptide levels greater than 0.2 pmol/mL at the time of diagnosis, although many of the subjects presented with acute metabolic syndromes involving ketoacidosis [5].

These findings provide a rationale for immune interventions that may preserve residual insulin secretion after onset of disease because clinically significant levels of insulin production remain in most patients. Moreover, the DCCT suggested that retention of insulin production was a valuable endpoint for treatment of T1D because improved metabolic control reduced the development of end-organ complications. Nonetheless, over time, C-peptide responses are impaired severely in most patients. In 73 adolescents with diabetes for more than 5 years, none had substantial insulin secretory capacity [4]. Even in adults initially thought to have type 2 diabetes mellitus who have markers of autoimmunity, progression to insulin-dependence is common [6].

Clinical goals and endpoints of trials in type 1 diabetes mellitus

The clinical endpoint of absent C-peptide secretion requires considerable time to reach (particularly in older subjects) and may vary greatly in a population of patients with new-onset T1D, so that large sample sizes are needed for intervention trials that use this endpoint. A surrogate endpoint that reflects a pathogenic event in the disease process and changes with this process would be valuable in pilot trials to determine the most promising interventions for testing in larger trials.

The most clinically relevant surrogate endpoint in T1D is glycosylated hemoglobin (hemoglobin A1c), which reflects glucose control and is related directly to the risk of long-term complications. This endpoint, however, is affected by factors including patient resources and the pattern of care that the patient receives; patient practices and compliance, including diet and exercise patterns; other social factors; and age. In the DCCT, hemoglobin A1c levels were higher systematically in adolescent patients, the age group with a high incidence of new-onset T1D. Moreover, glycosylated hemoglobin does not reflect the pathologic process directly, although there is general agreement that patients with higher rates of endogenous insulin production have more stable metabolic control. The same problems exist with insulin
dosage because it can be affected by patient compliance, as well as body weight and insulin sensitivity. Thus, either glycosylated hemoglobin or insulin dosage alone is likely to be problematic as a surrogate endpoint.

Autoantibodies are found in most patients with new-onset T1D, and identify prediabetic individuals at risk for the disease. After disease onset, the titers and frequency of the autoantibodies decline variably with time. Longitudinal studies of anti–glutamic acid decarboxylase (anti-GAD) antibody have reported a slow decline in their prevalence, from 82% at disease onset to 32% in 105 individuals with a median duration of disease of 21 years [7,8]. Some studies have suggested that autoantibodies may identify patients with T1D with a rapid decrease in C-peptide level after clinical onset [9,10]. In discordant first-degree relatives of patients with T1D, autoantibodies were found to identify persons in whom autoimmunity was active and in whom the risk for progression to diabetes was increased. Among the control subjects in the Canadian-European Cyclosporin trial, glucagon-stimulated C-peptide levels were more than 30% lower in glutamic acid decarboxylase (GAD) antibody–positive individuals compared with GAD antibody–negative individuals, but others have not seen this relationship and some have reported the converse with anti-GAD65 antibodies [7,11]. Indeed, in the recently reported Diabetes Prevention Trial-1 (DPT-1), the presence of islet cell autoantibodies (ICA) with anti-insulin antibodies and subtle impairment in first-phase insulin responses to an intravenous glucose challenge identified a subpopulation of first-degree relatives of patients with T1D in which 60% developed disease within 5 years.

Nonetheless, autoantibodies do not seem to be valuable as endpoints in immune intervention trials. Bougneres and colleagues [12] reported in the French Cyclosporin A trial that reduction in insulin requirements did not correlate with anti-GAD or other anti-islet autoantibodies. In a trial of azathioprine and prednisone, Silverstein and colleagues [13] reported that parameters including age of onset, metabolic status at trial onset, and the degree of lymphopenia correlated with response to treatment, but immunologic markers did not correlate. In the Canadian-European Cyclosporin trial, anti-GAD65 antibody titers did not change over time in either the Cyclosporin A–treated or control groups and the presence or absence of autoantibodies did not predict non–insulin-requiring remission in either group [11,14]. In a study of non–FcR-binding anti-CD3 monoclonal antibody (mAb), Herold and colleagues [5] reported that changes in the titer or isotype of autoantibodies did not predict clinical response to anti-CD3 treatment. In fact, there was little change in these autoantibodies over the first year of disease. In a small study of individuals at high risk for T1D (the Schwabing Insulin Prophylaxis Pilot Trial) Fuchtenbusch and colleagues [15] reported that treatment of seven high-risk individuals with insulin delayed the onset of T1D. The titers of ICA, antibodies to GAD, and tyrosine phosphatase–like protein islet antigen 2 remained unchanged, however. Interestingly, even high-dose glucocorticoid treatment of stiff-man syndrome, which is
associated with high titers of antibodies against GAD65, led to improved clinical status but failed to change the titer or epitope recognition of the anti-GAD65 antibodies [7].

Thus, although autoantibodies are markers of the disease and may predict its clinical course, there is little evidence that these immunoglobulins change with interventions that affect the natural history of the disease. This may be because the immunoglobulin response is matured fully by the time of diagnosis, and cannot be affected by changes in T-cell responses, which is the goal of immunosuppressive therapies.

Most experimental evidence suggests that T cells or their products are responsible for the islet damage and destruction in T1D. In general, conventional T-cell assays that measure proliferative responses to antigens have not been reliable or reproducible, however [16]. Two newer approaches may be more informative. A recent report from the first International Nonobese Diabetic (NOD) Mouse T-Cell Workshop concluded that use of enzyme-linked immunoabsorbant spots (ELISpots) to detect individual antigen-reactive T cells was a more sensitive assay to detect autoreactive cells [17–19]. For example, the DiaPep 277 pilot study reported that treatment with DiaPep preserved C-peptide responses and was associated with a T_{H1} to T_{H2} T-cell shift detected by ELISpot [20]. In addition, fluorochrome-labeled major histocompatibility complex (MHC) tetramers, which are aggregates of four MHC molecules with peptide, may enable investigators to directly enumerate antigen-reactive T cells [21].

This approach has shortcomings in identifying Class II restricted cells (the restriction element for autoreactive CD4^+ T cells). First, the studies can be done only in individuals with certain HLA types, and the antigenic peptide must be identified. In addition, because of the low precursor frequency of antigen-specific CD4^+ T cells in the peripheral blood, the antigen-reactive cells first must be expanded in vitro. Therefore, failure to identify tetramer-positive cells may be caused by reduced numbers of cells or by a functional failure to expand in cultures. A recent study in NOD mice used a Class I MHC tetramer to identify pathogenic CD8^+ T cells without the need for expansion in vitro, but the relevant CD8^+ T cells and their peptides in human disease are not known. Finally, a limitation of all approaches used to enumerate pathogenic T cells is that the assays use samples of peripheral blood. The cells in the peripheral blood may not reflect the populations at the sites of pathology. In the NOD mouse, for example, insulin reactive CD8^+ T cells could be detected in the islets of mice developing diabetes, but were barely detectable above background levels at other lymphoid sites [22]. (See the article by Tree and Peakman elsewhere in this issue for further exploration of this topic.)

In summary, because of its relationship with glucose control, the clearest endpoint for studies of T1D is a measurement of insulin production. It has not been established whether there is an optimal approach to evaluate insulin production or whether the different stimuli that have been used to trigger
insulin production (eg, glucose, glucagon, mixed meal) are comparable. Moreover, because of the heterogeneity of responses among individuals, only prospective evaluation and comparisons between individuals in treatment and untreated control groups will provide useful information. For use in study design and power calculation, unfortunately few prospective studies have characterized the loss of insulin production in T1D [23,24]. More prospective data would be valuable.

**Ethical issues in studies of patients with type 1 diabetes mellitus**

Unique issues surround the equipoise of trials in T1D, many of which have been addressed recently with the publication of guidelines for trials of patients with newly diagnosed T1D [25]. First, the balance of risks and benefits for interventions is changing because the long-term prognosis of the disease has improved in the last decade. Technologic advances have enabled more normal control of glucose levels for longer periods of time than was possible previously. Improved glucose control, based on the DCCT findings, would be expected to reduce rates of severe end-organ complications [26]. Indeed, recent reports have documented the declining rates of end-stage renal disease among patients with T1D, associated with improved metabolic control [27]. Thus, the tools now available for clinical management of diabetes with conventional approaches are better now than they were 5 years ago. Therefore, the improvement in glucose control and quality of life resulting from immune interventions must be substantial to warrant the potential risks. On the other hand, the goals established in the DCCT commonly are not met, particularly among adolescents, and when they are, it is with a threefold to fourfold risk of severe hypoglycemia. Therefore, the potential benefits of interventions that alter the primary disease process should be compared with realistically achievable treatment results.

Second, trials of T1D usually involve children. The disease can occur at any age, but the peak incidence is between 6 and 12 years of age. In testing of experimental therapies, particularly in phase-I trials, the inclusion of children adds concerns about the acute and long-term effects of the drugs. Chronic, broad-spectrum immunosuppression generally has been believed to involve too great a risk for infection, malignancy, and other side effects to warrant its use. The potential additive effects of complications from the disease and toxicities of the drugs (eg, renal toxicity from Cyclosporin A) are a unique problem. Study design issues involving the use of children need particular attention, including the use of placebo-controlled trials, particularly if hospitalization is required, and the ability of the subject to evaluate potential risks and benefits and give informed consent.

**Therapies directed at β cells**

Injury to islet cells may initiate the development of T1D, and repeated injury, through immune or other mechanisms, seems important in the
progression of disease [28]. Therefore, agents that would reduce islet cells’ susceptibility to damage may be of benefit for treatment. Nicotinamide was found to increase β-cell resistance to toxic chemicals and macrophage-mediated damage, and to increase β cells’ regenerative capacity (Fig. 1) [29]. Nicotinamide was shown to affect ADP-ribosylation reactions in β cells as well as in immune cells. β-Cell death was prevented and the phenotype of the immune response to islet antigens altered [29,30].

Several trials have tested the ability of nicotinamide to preserve insulin production in patients with new onset T1D. These trials involved nicotinamide as a single agent at a range of dosing as well as in combination with other immunosuppressive agents and intensive insulin. [31–36]. Initial trials suggested improved C-peptide responses following treatment with nicotinamide, but other studies failed to show a clinical benefit [31,32,34,36]. A meta-analysis of the trials indicated a significant effect of nicotinamide on C-peptide responses across the trials, even if only the trials with placebo control groups were considered. There was, however, no effect on glycosylated hemoglobin or insulin dosage. Thus, it was difficult to demonstrate repeatedly a clinical effect of nicotinamide alone or in combination with other treatment approaches in individual studies. This was the background

Fig. 1. Postulated sites of action of agents in recent and past trials for treatment of T1D. Animal models of T1D have suggested a dynamic interaction between effector T and T regulatory cells in the destruction of β cells, which may be affected by a number of treatment strategies (italicized text). APC, antigen-presenting cells; ATG, antithymocyte globulin; AZA, azathioprine; CsA, Cyclosporin A; GC, glucocorticoids; MMF, mycophenolate mofetil; Pred, prednisone.
for a diabetes prevention trial (European Nicotinamide Diabetes Intervention Trial) that ultimately showed no beneficial effect of nicotinamide treatment on prevention of disease [37].

Insulin treatment itself may have beneficial effects on the retention of insulin production. Madsbad and colleagues [38] reported that in patients with fasting C-peptide levels greater than 0.07 nmol/L, improvement in β-cell function occurred during a period of strict blood-sugar control, but improvement disappeared 3 weeks later. This suggested a rapid but reversible effect of glucose control on β-cell responses. In a small randomized trial, Shah and colleagues [39] found that administration of insulin intravenously for 2 weeks using an artificial pancreas (Biostator) at a dose four times greater than conventionally treated subjects resulted in higher stimulated C-peptide levels and improved hemoglobin A1c levels 1 year after treatment. Data from the DCCT, involving patients with T1D for 1 to 5 years with residual C-peptide (C-peptide responders) followed for 4 years, showed that patients assigned to the intensive treatment group had higher C-peptide levels and a lower rate of becoming a C-peptide nonresponder than those assigned to conventional treatment, which involved less aggressive control of glucose [4]. In the DCCT, however, patients were maintained continuously in their respective glycemic treatment group, so that the duration of any beneficial effect could not be studied.

There are a number of possible reasons for a beneficial effect of insulin. β-Cell function may improve by eliminating the toxic effects of glucose. Insulin-induced rest of β cells may reduce expression of the islet antigens or reduce susceptibility to damage. In addition, murine studies suggest that insulin may promote β-cell development. Insulin signaling and activation of insulin receptor substrate (IRS)-2 is important for β-cell development because mice lacking IRS-2 have a 50% reduction in β-cell mass [40,41]. The failure of β-cell redevelopment and differentiation in T1D may be caused in part by the absolute deficiency of insulin. The relationships between insulin secretion and responses to immune therapies have not been well studied, but seem to be important. For example, improved responses to treatment with Cyclosporin A occurred in individuals with higher levels of C-peptide at disease onset [12].

Trials of broad-spectrum immunosuppressive drugs in type 1 diabetes mellitus

Cyclosporin A was tested in early immune intervention trials. Cyclosporin A blocks cytokine production by all T cells, limiting expansion of the T-cell pool and preventing the secretion of cytokines, thought to be important direct mediators of β-cell destruction, such as interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α) [42,43]. Preclinical studies in the NOD mouse and biobreeding/Worcester rats showed that Cyclosporin A could prevent the development of diabetes [44,45]. Immune intervention trials in T1D began in
1984 with a pilot trial of Cyclosporin A in which 16 of 30 patients treated within 6 weeks after diagnosis became insulin-independent with C-peptide levels in the normal range [46]. This experience was confirmed in a trial from France in 1988 and supported by studies from the University of Miami in 1992 [12,24]. In the French trial, 27 of 40 Cyclosporin A–treated individuals were able to discontinue insulin 48 days after initiating drug treatment and maintained an average hemoglobin A1c level of 6.15%. Seventy-five percent of the 27 patients remained off insulin 1 year after initiating treatment. Immunologic factors such as HLA type and autoantibodies did not predict response to immune treatment. In contrast, clinical parameters such as ketoacidosis and weight loss, mirrored by higher C-peptide levels in clinical responders, did predict response.

The effects of Cyclosporin A on the initial stages of the disease were dramatic, but the long-term effects and toxicities raised questions about adopting this therapy for more widespread use. De Filipo and colleagues [47] found that after stopping treatment with Cyclosporin A in patients 6 to 62 months after diagnosis, C-peptide levels initially were higher than in untreated patients, but the improvement disappeared after 4 years. Although the treatment effect lasted beyond the period of drug administration, those authors believed that the potential risks associated with Cyclosporin A did not justify its use for treatment of new-onset T1D. Of greater concern was the possibility that renal toxicity was induced in patients who had received Cyclosporin A. In a group of 21 patients treated with Cyclosporin A for 12.5 months (±4 months), four developed microalbuminuria, and overall 24-hour albumin excretion rates were higher in Cyclosporin A–treated patients compared with diabetic control subjects after 7 years [48]. This experience has not been confirmed in other studies of patients with T1D treated with Cyclosporin A, however [49,50]. Nevertheless, because it is not possible to identify at the time of diagnosis individuals who may eventually develop renal disease, the risks of renal disease associated with use of Cyclosporin A create concern for its use.

Although Cyclosporin A targeted cytokine production, other broad-spectrum immunosuppressive regimens also may be effective in preventing the loss of insulin production. In a randomized, controlled trial, Silverstein and colleagues [13] reported that patients with new-onset T1D receiving glucocorticoids daily for 10 weeks and azathioprine daily for 1 year had improved C-peptide responses compared with untreated control subjects. The drug toxicities, including hair loss, gastrointestinal upset, and Cushingoid appearance, however, reduced enthusiasm for this approach. Buckingham and Sandborg [51] conducted a randomized trial of methotrexate in 10 patients with newly diagnosed T1D but failed to show improvement in fasting or stimulated C-peptide responses.

Eisenbarth and colleagues [52] tested a strategy directed toward T cells by giving subjects antithymocyte globulin with glucocorticoids at the time of disease onset. In a small pilot trial, they reported that drug-treated patients
had lower hemoglobin A1c levels while requiring reduced doses of insulin for at least 100 days after drug therapy, and two of the five patients did not require insulin for more than 8 months. Severe thrombocytopenia occurred in two patients, however, and it was believed that the risks of this drug treatment did not offset its potential benefits.

Most tested broad-spectrum immunosuppressive regimens showed short-term efficacy but required continuous administration to maintain an effect; none of the agents induced tolerance, a condition in which the immunosuppressive therapy could be withdrawn without recurrence of the immune response [53]. Moreover, enthusiasm waned in the 1990s because of concerns including toxicities; insulin resistance induced by glucocorticoids, which interfered with attempts to maintain normal glucose control; the potential risk for malignancies; and cosmetic issues. It also was believed widely that the residual \( \beta \)-cell mass present at the time of diagnosis of T1D was too small to be of clinical significance, and the chance of significant recovery unlikely, so that the additional risks of the immune drugs were unwarranted. Furthermore, with the improved technology for clinical care, new interventions for T1D would require a better safety profile than conventional immunosuppressive agents, and would not require lifelong treatment. Induction of tolerance has emerged as the goal for immune therapies.

**Antigen-specific immunotherapy**

The identification of disease-specific antigens in T1D has been a major development of the past decade. These discoveries led to hope to induce tolerance that would inactivate or affect the specific pathogenic T cells recognizing the antigens, which may be triggering the autoimmune response. Antigen-specific approaches have shown efficacy consistently in preclinical studies in the NOD mouse. These strategies are based on the understanding that a response to antigen is affected by factors including the strength of the antigenic signal, costimulation, and the cytokine environment. Therefore, by modulating these parameters, it may be possible to divert pathogenic responses to the antigens into a protective, nonpathogenic response [43]. In addition to modifying the strength of the T-cell receptor signal with altered ligands or adjuvants, other investigators have administered antigens orally, intranasally, or using hematopoietic stem cells to alter the presentation of antigens or induce immune regulation to the administered antigens [54–57]. Several antigens that have been tested in preclinical studies are now in development for clinical trials.

Treatment of NOD mice with parenteral or oral insulin will prevent diabetes [54,58]. Administration of GAD65 to NOD mice prevented diabetes and delayed its recurrence in syngeneic islets transplanted into diabetic mice. Vaccination of NOD mice with GAD65 modulated the balance of \( T_{H1} \) and \( T_{H2} \) responses to the antigen [59]. Vaccinating NOD mice with an
altered-peptide ligand based on the immunodominant autoantigen on insulin B-chain (9–23) stimulated interleukin (IL)-4 and IL-10 production to the altered peptide and the native antigen. This stimulation suggests that the immunization skewed the pathogenic response toward a nonpathogenic response [60]. The IL-4 produced may create an environment that favors development of nonpathogenic effectors.

This understanding of the mechanism of vaccination with antigen may be an oversimplification, however. The secretion of IL-4 by T cells may be the result and not the cause of immune regulation. For example, NOD mice genetically deficient in IFN-γ are not resistant to the development of diabetes, and IL-4– or IL-10–deficient mice do not develop accelerated disease [61]. In addition, this explanation for the effects of immunization does not take into account the role of other non–T cells that are involved in the protection from diabetes induced by Th2 cytokines [62]. Finally, a note of caution about the effects of diverting antigen responses has been raised. Fatal anaphylactic responses to both GAD65 and insulin have been induced in NOD mice by vaccination with self-peptides [63,64].

Preclinical studies indicate that immunization of NOD mice with BCG vaccine could prevent disease onset and recurrence in syngeneic islet grafts [65,66]. Although the response to the immunogen is directed toward an antigen that seems to be irrelevant to the disease, the mechanism of action was thought to involve a shift in immune responses from a destructive Th1 to a protective or nonpathogenic Th2 response because increased levels of IL-4 were seen in draining lymph nodes from immunized mice. Despite the clear therapeutic effects in the NOD mouse, however, two double-blind, controlled studies of BCG vaccination in patients with new onset T1D failed to show an effect on the decline in C-peptide responses over the first 18 months and 2 years of disease [67,68].

Despite the promising results of insulin immunization in mice, the results from a human trial of oral insulin in recent-onset T1D (the IMDIAB VII study) were disappointing. In the study of 82 patients with T1D of less than 4 weeks’ duration, oral administration of insulin (5 mg/d) had no effect on basal C-peptide and hemoglobin A1c levels after 3, 6, and 12 months of treatment. There was a tendency for lower C-peptide values at 9 and 12 months in patients less than 15 years of age [69]. Studies to evaluate the effects of immunization with modified insulin peptide are in progress. (See the article by Eisenbarth and Jasinski elsewhere in this issue for further discussion of this topic.)

Immunization of mice with a peptide of heat shock protein (HSP) 60 was shown to prevent autoimmune diabetes in two murine models of human disease [70,71]. HSP60 peptide p277 is a postulated autoantigen in T1D [20,72]. Studies from the NOD mouse have indicated that vaccination with HSP60 may establish regulatory circuits resulting in reduced expression of pathogenic cytokines and increased expression of protective ones [73]. In a randomized, controlled, and blinded study of 35 patients, Raz and
colleagues [74] found that stimulated C-peptide levels in patients with T1D were increased following vaccination with 1 mg of p277 HSP60 peptide. The immune responses to the peptide (but not to other antigens) showed an enhanced Th2 phenotype. Moreover, repeated vaccination with the peptide controlled relapses, and these vaccinations were reported to be free of side effects. Importantly, immunization with this ubiquitous antigen has been the first evidence that antigen-specific vaccination may be effective in treating T1D. Further studies with this reagent are in progress.

Other approaches

Orally administered IFN-α is being studied for treatment of T1D. The postulated mechanism of action involves a reduced expression of cytokines thought to have direct toxic effects on β cells: IL-1, TNF-α, and IFN-γ. Sobel and colleagues [75] reported that oral administration of IFN-α prevented the development of diabetes in the NOD mouse. The oral cytokine did not cause general immunosuppression, but had an indirect effect on effector cells and could be broken by adoptive transfer of the cells. Other studies indicated that oral IFN-α prevented but did not reverse diabetes once hyperglycemia had developed [76].

In other clinical settings, however, the effects of IFN-α on T1D have conflicted with this benefit. IFN-α has been identified in the islets of patients in an analysis of biopsied pancreatic tissues at the time of presentation of diabetes. Parenteral IFN-α was shown to worsen glucose control in patients with T1D and hepatitis C infection, and to precipitate diabetic ketoacidosis in patients with hepatitis C [77–80]. In contrast, Brod and colleagues [81] reported that ingested IFN-α clinically improved relapsing multiple sclerosis, which shares pathogenic features with T1D. These studies suggest that oral IFN-α reduces levels of soluble intercellular adhesion molecule, a disease marker, and secretion of IL-2, IFN-γ, IL-10, and transforming growth factor (TGF)-β from concanavalin A–stimulated lymphocytes [82]. In a small pilot study, Brod and colleagues treated 10 newly diagnosed T1D patients with 30,000 IU ingested IFN-α within 1 month of diagnosis and examined the difference between baseline and stimulated C-peptide responses every 3 months during the first year of disease [83]. Eight of the 10 patients showed preserved β-cell function, with at least a 30% increase in stimulated C-peptide levels at 0, 3, 6, 9, and 12 months after initiation of treatment. There was no discernible chemical or clinical toxicity associated with ingested IFN-α. Controlled studies with this agent are now in progress.

In 1994, Chatenoud and colleagues [84] reported the striking preclinical finding that treating NOD mice with anti-CD3 mAb when the mice first presented with hyperglycemia could reverse the hyperglycemia in approximately 80% of treated animals. Continuous immunosuppression was not needed; 5-day treatment reversed hyperglycemia 2 weeks later, and the
disease did not recur for more than 6 months. In diabetic mice treated with anti-CD3 mAb, syngeneic islets could be transplanted without recurrence of disease or continuous administration of the mAb [85]. Thus, anti-CD3 mAb induced true tolerance to autoimmune diabetes in this model.

Studies of the mechanisms in the NOD mouse suggest that mAb may induce regulatory cells or regulatory phenomena [86]. Diabetogenic cells remained but were inactive in the treated animals; the transfer of spleen cells from treated mice into untreated recipients transferred diabetes, but treated mice were resistant to such transfer of diabetes. Treatment of mice with Cyclosporin A at the time of mAb treatment prevented reversal of diabetes, leading to the belief that T-cell activation or cytokine production is required for the effect.

The mouse antihuman CD3 mAb, OKT3, was developed originally for treatment of transplant rejection. A number of problems with the drug precluded its use in patients with T1D, however. OKT3 causes T-cell activation in vivo, resulting in the release of cytokines (the cytokine release syndrome) and clinical toxicity including fever, hypotension, myalgias, and arthralgias [87,88]. Patients treated with the murine mAb frequently develop a human antimouse immunoglobulin response that can neutralize the mAb and preclude retreatment.

To eliminate the in vivo T-cell activation induced by anti-CD3 mAbs, Bluestone and colleagues eliminated the FcR-binding portion of anti-CD3 mAb and studied the effects of F(ab’)2 anti-CD3 mAb in murine systems. The authors and others showed that F(ab’)2 fragments of anti-CD3 mAb could cause immune modulation in diabetes and other immune settings, without the T-cell activation in vivo and morbidity in the recipients [89–91]. Additional preclinical studies suggested that by eliminating the FcR-binding portion of the anti-CD3 molecule, the T-cell activation signal and effects on T cells were different than FcR-binding anti-CD3 (like OKT3). The non–FcR-binding mAb induced anergy of previously activated T cells, but naive cells were unaffected [92,93]. In addition, the inhibitory effects were limited to previously activated T H1 cells, thought to be involved and present in the islets of patients with T1D [94]. Thus, preclinical studies in models of T1D indicated that anti-CD3 mAb may induce tolerance to autoimmune diabetes, and modifications of the anti-CD3 molecule seem to eliminate the toxicities associated with anti-CD3 mAb and change the actions in a manner that could induce tolerance to the disease.

Xu and Bluestone [95] synthesized a humanized non–FcR-binding anti-CD3 mAb, hOKT3γ1(Ala-Ala), with the intention of developing a new reagent that would not have the same activation properties in vivo of OKT3 and to which human immune responses would be limited. It was tested initially in a trial of renal and renal/pancreas allograft rejection. The response rates with the humanized antibody were similar to OKT3, but the side effects and cytokine release syndrome were minimal [96]. Similar findings were seen in a phase-I trial of the drug in patients with refractory Psoriatic arthritis [97].
In 1999, the authors initiated a phase-I/II, controlled randomized trial of hOKT3γ1(Ala-Ala) in patients with new-onset T1D. Treated patients received 10 days of a full dose of hOKT3γ1(Ala-Ala) preceded by a 2- or 4-day ramp in dosing. C-peptide responses to a mixed meal were measured every 6 months for 2 years, and the total area under a 4-hour response curve (AUC) was compared in the two groups. Metabolic management was similar in both groups, and the drug was well tolerated. The principal side effects included fever in 75% and rash in 83% of subjects. A mild and brief cytokine release syndrome consisting of headache, nausea, vomiting, and arthralgias occurred in less than one third of patients, generally for 1 day after the first full dose of drug. On the day following the first full dose, the nadir lymphocyte count of approximately 25% of the starting lymphocyte count was reached, but the lymphocyte count later increased, so that by 2 weeks after the last dose of drug, it was at 123% of pretreatment levels.

One year after enrollment, the mean AUC in the drug-treated group was 97.1% of the baseline value, whereas in the control group it was 53.1% of the starting value \( (P < 0.001) \) [K.C.H., unpublished data, 1999]. After 1 year, three quarters of patients had stable or improved C-peptide responses in the drug-treated group compared with one sixth of control subjects \( (P < 0.01) \). The improvement in C-peptide response was accompanied by significant improvement in metabolic control, as reflected by hemoglobin A1c levels and reduced use of insulin in the treated group \( (P < 0.001) \). Thus, the anti-CD3 mAb caused persistent improvement in insulin secretion, and the accompanying metabolic improvement, without the need for continuous immune suppression. Beyond the first year after enrollment, the average C-peptide response in the treated and control groups declined, but the AUC responses in the treated group were still significantly greater.

None of the conventional markers of autoimmunity (e.g., anti-GAD or anti-ICA512 antibodies) could distinguish clinical responders from non-responders. Instead, compared with nonresponders, clinical responders to the drug treatment had an increase in the relative number of CD8\(^+\) T cells compared with the number of CD4\(^+\) T cells. This change was caused by an increase in the number of CD8\(^+\) T cells that repopulate the periphery after the transient depletion of T cells following drug treatment, rather than a decrease in the number of CD4\(^+\) T cells.

Further studies suggested a different mechanism of drug action from those previously described. The non–FcR-binding anti-CD3 mAb delivered an activation signal to T cells, resulting in a relatively greater release of IL-10 compared with IFN-\(\gamma\) than occurs with conventional anti-CD3 mAb [98]. This mechanism is consistent with the detectable levels of IL-10 that were found in the serum of patients after drug treatment and in previous studies in transplant patients [96]. In addition, the authors identified a subpopulation of CD4\(^+\) CCR4\(^+\) CD45RO\(^+\) circulating T cells at the end of mAb treatment that seemed to be producing IL-10 in vivo. The role of these cells...
is unclear, but several lines of evidence suggest a regulatory role for IL-10 and IL-10+ CD4+ T cells [99–101].

Thus, this first pilot study of anti-CD3 mAb in patients showed that genetic modification of the mAb may reduce the side effects seen with activating anti-CD3 mAb and may have long-lasting effects without the need for continuous administration and immunosuppression. Additional trials with this and other anti-CD3 antibodies are in progress.

Summary

Although T1D is a chronic autoimmune disease that eventually leads to complete organ loss, because it presents when clinically significant function is still present, there is an optimal window for interventions that may slow or even reverse the progression of disease. Moreover, studies in animal models of the disease as well as in patients and their relatives have provided insight into the disease mechanisms and suggested approaches, such as the induction of tolerance so that continuous immunosuppression is not needed. Translation of the developments in experimental diabetes to clinical trials has been slow, however. A surrogate marker of the disease process would be valuable in speeding this translation, but the search for a surrogate marker for the decline in insulin secretion, the hallmark of the disease, has not been successful. Early clinical studies have shown short-term benefits, but have been limited by (1) toxicities of the agents and side effects from their use, (2) the need for continuous immunosuppression, (3) nonspecific effects on immune function, and (4) the lack of long-lasting benefits. Newer strategies, some of which have already been tested in pilot studies, may overcome these shortfalls, and trials over the next several years will determine whether these approaches can achieve the goal of specific tolerance.

Finally, although the insulin secretion present at diagnosis is clinically important, complete correction of diabetes will require additional replacement of islet cells, and immune protection will be needed so that the new cells do not succumb to the fate that led to the disease. Islet transplantation has become feasible, and the immunologic issues surrounding replacement of islet tissue are similar to those in the primary autoimmune disease. The renewal capacity of β cells once autoimmunity has been arrested is not known, but is suggested by the finding of increased C-peptide responses in patients after anti-CD3 mAb treatment, in the NOD mouse following induction of tolerance, and by the persistence C-peptide responses after successful islet transplants [102–104]. A particularly attractive approach is to differentiate β cells from precursor stem cells, so that a supply of cells will no longer be limiting. Indeed, recent studies have shown the regenerative capacity of ductal cells and even bone marrow–derived cells [105,106]. Whether newly formed islets will succumb to the same autoimmune attack as the original cells is not known. It is likely, therefore, that a combination of both cellular and
immunologic approaches to restore functional β-cell mass and induce tolerance to autoimmunity will be needed to successfully reverse of the disease.

References


