INTRODUCTION

Though it is possible today to predict the development of type 1 diabetes, we still do not have an effective and safe preventive therapy. The frequency of multiple islet autoantibodies in general population is about 1/300, and one can predict that approximately one million individuals in the United States are at high risk of developing type 1 diabetes. Thus development of safe and effective preventive therapy for this disease assumes significance even from the public health perspective. One could consider trials for the prevention of type 1 diabetes at almost all stages of the disorder from genetic susceptibility to immunosuppression in patients with long-standing diabetes who receive an islet transplant. Trials at the onset of type 1 diabetes are the most common, and the primary goal here is preservation of beta cell function as reflected by C-peptide secretion.

The major issues involved in the preventive strategies for type 1 diabetes are effectiveness of prophylaxis, duration of the prophylaxis, complexity of the treatment regimen, and treatment-related toxicity. The Diabetes Control and Complications Trial (DCCT) confirmed that the microvascular complications of type 1 diabetes can be delayed by good metabolic control. Thus, the benefits, inconveniences, and toxicity of type 1 diabetes prophylaxis must be weighed against the potential for good control. Introduction of continuous glucose monitoring is likely to change the risk-benefit equation over the near-term. Adherence to a prophylactic regimen may well also require the same degree of compliance as does maintaining a good A1C level. An ‘ideal’ prophylactic therapy needs to be administered for a limited time but should have life-long efficacy. While there are no secure data in humans on type 1 diabetes prevention as yet, there is an abundance of information from animal models on prevention of diabetes in non-obese diabetic (NOD) mice and BioBreeding (BB) rats. It is important to remember that many partially effective interventions that work in rodents have not, to date, worked in man. It is likely that both surrogate markers of therapeutic efficacy (e.g. assays of pathogenic T cells), more robust animal models (e.g. with 100% of animals developing diabetes), and more robust therapies (e.g. prevention not only of diabetes but of insulitis) will indeed be needed.

ANIMAL MODELS FOR TYPE 1 DIABETES PREVENTION

The weaknesses of the animal models (BB rats and NOD mice) stem mainly from our ignorance of the mechanisms by which autoimmunity is triggered and/or sustained in the experimental animals. In general, therapies are most effective when administered early (e.g. prior to onset of insulitis) than when administered at onset of type 1 diabetes disease. It is hypothesized that therapies that either eliminate insulitis completely or are effective in more robust animal models will better predict success in man.

CLINICAL TRIALS FOR THE PREVENTION OF TYPE 1 DIABETES

The recognition of insulin-dependent diabetes mellitus as an immunological disease led to the first controlled trials of interventions using nonspecific immunosuppressive treatments in subjects already diagnosed with diabetes. Cyclosporine A (CyA) and prednisone-azathioprine were tried initially because they had been effective in animals. These early trials showed that progressive loss of insulin secretion (as measured by C-peptide assays) could be delayed for a limited period of time while immunosuppression was maintained, and the natural progression of the disease was reinstituted following withdrawal of immunosuppression.

Prophylactic trials in subjects with new/recent-onset diabetes are based on our current understanding that beta cell destruction is a gradual process and that an intercurrent stress precipitates loss of glucose homeostasis and symptoms of hyperglycemia before all the insulin secretory capacity is lost. Eighty to 90 percent of islets would have already been destroyed by the time diabetes is diagnosed. Thus, the best possible outcome of trials in people who already have diabetes is to prolong the period when small amounts of insulin continue to be produced, rather than complete reversal of diabetes. Benefit to the subject under these circumstances might come not from discontinuing insulin injections but from smoother day-to-day blood glucose control, lowering A1C levels, and reducing the risk of hypoglycemia and diabetes-related complications. Benefit of this type is unlikely to appeal in people with recent-onset diabetes unless the prophylactic regimen is
simple, safe, and/or long-lasting with a single course of therapy. Measurement of C peptide level is the most reliable test of endogenous insulin secretion in insulin-requiring individuals. Loss of C-peptide may occur over a number of years, and the rate of loss is usually much more rapid in children compared to adults with type 1 diabetes. A better preserved C-peptide is associated with improved metabolic control with a lesser dose of insulin. It will be critical to have data from long-term follow up of patients with preserved C-peptide with continuous glucose monitoring to determine parameters to power trials to decreasing hypoglycemia as a potential secondary endpoint. Most investigators recommend preservation of C-peptide as the primary endpoint in new-onset trials coupled with secondary endpoints of improved A1C and decreased glucose variability and in particular decreased hypoglycemia.

Another approach for clinical trials is to enroll 'prediabetic' individuals. The best possible outcome in prediabetic individuals would be the maintenance of insulin production and a life free of insulin replacement. It is now possible to ascertain individuals with a higher risk of developing diabetes both for relatives of type 1 diabetes subjects and the general population. The yield of prediabetics (about 6% of those relatives screened expressing an anti-islet autoantibody) is small, and the prevention trials completed to date such as DPT-1 (Diabetes Prevention Trial Type 1) have screened more than 100,000 relatives of subjects with type 1 diabetes. Trials with 'at-risk' individuals being very labor-intensive, prevention trials in recent-onset cases are likely to continue to be important.

TRIALS IN NEWLY DIAGNOSED SUBJECTS

Cyclosporine A (CyA)

One of the earlier successful methods for prolonging insulin production has been administration of CyA. Unfortunately, the effects of CyA on T cells were lost when it was discontinued, and CyA is nephrotoxic; hence, additional studies with CyA alone, in subjects with newly diagnosed type 1 diabetes were not pursued. The impact of the trials with CyA, despite these negative conclusions, should not be underestimated; the initial promise of immunosuppression as a means to prevent diabetes stimulated interest in the early diagnosis of diabetes and led, ultimately, to screening approaches by which prediabetes could be recognized. The trials also stimulated awareness of the cost/benefit ratio of interventions to prevent diabetes.

Other Immunosuppressive Agents

An immunosuppressive regimen consisting of anti-IL-2 receptor monoclonal antibodies, rapamycin (sirolimus), and low dose FK506 (tacrolimus, a calcineurin inhibitor similar to cyclosporine A), achieved insulin-free status in patients receiving islet transplants for type 1 diabetes in a recent Canadian study. For the great majority of individuals, diabetes recurred with a few developing increasing anti-islet autoantibodies.

Mycophenolate mofetil (Cellcept), an inhibitor of the synthesis of the purine guanosine monophosphate (GMP) from inosine, prevents diabetes in BB rats. It appears to be more potent than azathioprine, and when used with calcineurin inhibitors, has had a major benefit allowing reduction in steroid dosage for pancreas transplantation. An unpublished trial of mycophenolate mofetil alone and in combination with anti-IL2 receptor antibody in patients with new-onset diabetes (as part of Trialnet study) did not influence loss of C-peptide. In a very small trial, methotrexate did not preserve C-peptide secretion in new onset patients.

Anti-CD3 Therapy

Monoclonal antibodies directed at the T cell receptor CD3 are able to reverse hyperglycemia in acutely diabetic NOD mice, but the same antibodies do not prevent diabetes when administered earlier in the course of disease. There is extensive data that the anti-CD3 therapy restores tolerance and induces regulation in NOD mice.

Multiple human trials are completed and phase III trials are underway in patients with recent onset diabetes using engineered anti-CD3 monoclonals (“humanized” and engineered to “abrogate” complement Fc binding). In contrast to standard anti-CD3 monoclonal antibodies, these antibodies do not induce severe cytokine release syndromes but have been associated with fever, rash, and in some patients, adenopathy depending upon the dose used. Several important studies indicate preservation of C-peptide secretion for one to two years and limited toxicity with a subset of patients developing anti-idiotypic antibodies. It appears that progressive loss of C-peptide secretion recurs after 12 months, though effects persist with a single course of therapy for up to 5 years. A concern is the reactivation of EBV infection in the European studies, but such reactivation was self-limited with a single course of therapy. With the available limited human data it is hypothesized that the larger term effects of anti-CD3 relate to induction of regulatory T-cells including potential CD8 regulatory T cells. It is likely that this form of therapy will require repeated administration of the monoclonal antibody and trials are planned with repeated courses of therapy in new onset patients. Combination therapy, particularly with antigen therapy is an important consideration.

Insulin Metabolic Therapy

Infusion of large quantities of exogenous insulin for tight blood glucose control has been reported to preserve C-peptide secretion post development of diabetes. The stress of keeping the patient constantly in bed for 14 days with a large needle in place would probably have prevented widespread acceptance of this treatment, and there has been no replication of these studies in the subsequent two decades. Trialnet study of parenteral insulin for diabetes prevention using annual intravenous insulin and daily modest dose of insulin did not delay progression to diabetes. The DCCT follow up data has also clearly demonstrated that intensive insulin therapy preserves C-peptide secretion.

Immunostimulation

Another approach to prolonging insulin production in the newly
diagnosed diabetes subject is through immunostimulation. This strategy is based on the observation that immunological stimulation of NOD mice with agents as diverse as allogeneic cells or the mycobacterial vaccine, bacillus Calmette-Guérin (BCG) lowered the incidence of diabetes. The mechanism for the protection is not clear; though stimulation and expansion of populations of T cells producing IL-4 or IL-10 has been suggested, and the mechanism of action may well be immunologically nonspecific.

Treatments to halt islet cell destruction and prevent diabetes would ideally be started before the beta cell mass is significantly compromised. Nevertheless, detecting partial benefit in recent-onset diabetics would be useful if it is a precursor to an intervention that could be used with greater efficiency in subjects with pre-type 1 diabetes.

**TRIALS IN SUBJECTS WITH PRE-TYPE 1 DIABETES**

National Institute of Health (NIH) Consensus Report on Prevention of Type 1 Diabetes concluded that there is indeed a methodology that can identify, with near certainty among first-degree relatives of type 1 diabetic patients, those who will develop diabetes and that immune intervention therapy before the onset of symptoms might prevent the disease from occurring. It is now generally accepted that prediabetes can be accurately diagnosed in select high-risk groups of subjects. The justification for identifying this high-risk group comes from their potential recruitment into protocols to test strategies to prevent diabetes as well as avoiding morbidity and the risk of death at onset; these high-risk subjects are, however, rare, and identifying prediabetics is very expensive, so a cost-effective approach requires extensive and international collaboration. The largest prediabetic prevention studies to date have evaluated the effects of insulin (parenteral, nasal, and oral administration) and nicotinamide.

The DPT-1 trial groups formed “TrialNet” in October 2001 to create a North American network for conducting trials for the prevention of type 1 diabetes and international sites were added later. TrialNet will evaluate therapies in new-onset patients as well as pre-diabetics and is open to suggested therapies and protocols. With such a collaborative network, it is hoped that the discovery of effective and safe therapies will be hastened. There are also additional efforts to develop therapies for diabetes prevention. Specifically, the Immune Tolerance Network (www.immunetolerance.org) is accepting applications to support therapies aimed at tolerance induction and assays of tolerance. The Immune Tolerance Network, sponsored by the NIH, funds studies both within and outside of the United States.

Nasal insulin was evaluated in a novel and ambitious study from Finland (DIPP - Diabetes Prediction and Prevention) where infants were followed from birth; when anti-islet antibody was found, the children were randomized to either nasal insulin therapy or placebo groups. Though the therapy did not delay progression to diabetes, ability to predict and conduct an excellent trial was clearly demonstrated. Another international study (TRIGR) is evaluating exclusion of bovine milk in early childhood with preliminary data. There is contradictory data relative to the epidemiologic analysis of bovine milk exposure related to diabetes risk with a recent manuscript reporting heterogeneity related to the PTPN22 genetic polymorphism associated with diabetes risk. The TRIGR trial is now fully enrolled and should provide a definitive answer as to importance of early introduction of bovine milk related to etiology of type 1 diabetes.

**ADDITIONAL CLINICAL TRIALS OF ANTIGEN BASED THERAPIES**

At least four islet antigens in addition to intact insulin have entered clinical trials for preservation of insulin secretion at diabetes onset: an altered peptide ligand of insulin peptide B:9-23 (Neurocrine), the GAD65 molecule (Diamyd), a heat shock protein peptide (Peptor), and the HLA-DR4-restricted proinsulin peptide C19-A3. A phase I/II trial is evaluating an altered peptide ligand of the immunodominant insulin B:9-23 peptide, a major target of intra-islet T cells. This peptide could, by multiple routes of administration, prevent diabetes. The peptide is identical in man and mice. However, there are several caveats relative to peptide immunotherapy. Even with a self-peptide, one can induce anaphylaxis; a self-peptide may activate disease, and insulin autoantibodies have been induced in normal Balb/c mice and NOD mice with the insulin B:9-23 peptide. The altered peptide ligand had no effect on loss of C-peptide in new onset trial.

The whole GAD65 molecule has entered clinical trials in Scandinavia in patients with Latent Autoimmune Diabetes in Adults (LADA), and a completed phase II study in new onset type 1 diabetes has been reported. Demonstration of preservation of C-peptide secretion by GAD65 immunization is a difficult task. At one dose (20µg) in the phase II study there was reported significant preservation of C-peptide. Injections did not have side effects and in particular there is no evidence of induction of Stiff Man Syndrome. A controlled trial in new onset patients with type 1 diabetes with a single injection of GAD65 in alum demonstrated decreased loss of C-peptide secretion but without effect on either A1C or insulin utilization.

**CELLULAR THERAPIES**

A number of approaches are being pursued in terms of cellular therapies to prevent beta cell destruction. Initial human trials are planned; in particular, there is a large preclinical effort to utilize regulatory T cells to modulate autoimmunity as well as autologous stem cells and antigen presenting cells.

There is a large body of evidence demonstrating the importance of regulatory T cells for the natural prevention of type 1 diabetes. With the rare mutation of the foxP3 gene that controls development of a major subset of regulatory T cells it is estimated that 80% of children develop type 1 diabetes in addition to multiple other autoimmune disorders. In preclinical studies regulatory T cells have been expanded and are able to prevent diabetes of the NOD mouse. Human regulatory T cells can be expanded in...
vitro though their stability as a regulatory T cell is questioned. Initial human trials will likely utilize mixtures of regulatory T cells, though antigen specific T cells provide greater efficacy in preclinical studies. Another proposed cellular therapy utilizes dendritic cells, either pulsed with antigen or modulated in vitro to be tolerogenic. It is likely that the initial clinical trials will utilize dendritic cells without antigen pulsing.

CONCLUSIONS

The fact that complications of type I diabetes can be delayed by good glycemic control has placed significant constraints on treatments intended to prevent the disease. Global immunosuppression protocols have an unacceptable morbidity. There is a need to establish organ-specific immunological tolerance. The approaches that have been explored to date in large preventive trials seem very speculative when viewed in relation to the complexity of immunoregulation and the development of autoimmunity. The lack of surrogate markers for the desired therapeutic T cell effects complicates interpretation. Potential surrogate markers for T cells are just becoming available for the NOD mouse and man. Until they are available, clinical studies of diabetes prevention will be difficult. However, the limitations of animal models necessitate the development of prevention approaches in humans. Constraints on research funding have increased the pressure for collaboration beyond local and national boundaries. The initiation of TrialNet and large multi-center trials (e.g. Immune Tolerance Network) to determine whether type I diabetes can be prevented or ameliorated at onset promises to make this decade an exciting one.

REFERENCES