Symposium Summary, Experimental Biology 2001

Marc S. Hurlbert, James F. Stallard, and Richard W. Furlanetto

INTRODUCTION

Type I diabetes is a major health problem, affecting more than 1.5 million Americans, and advances in therapy for this disease are of interest to both physicians and patients. In the past few years, significant advances have been made in our understanding of the pathogenesis, prevention, and potential cure of this disease. These advances formed the theme of a symposium held at Experimental Biology 2001 in Orlando, Fla, which was sponsored by the American Federation for Medical Research and the Juvenile Diabetes Research Foundation International (JDRF). The symposium opened with Dr Richard Furlanetto of the JDRF introducing the speakers and topics of the session. The session began with Dr George Eisenbarth discussing the pathophysiology of type I diabetes. Dr Dale Greiner then discussed immune tolerance, a potential target for both treatment and possible prevention of this autoimmune disorder. Finally, Drs Mark Atkinson and James Shapiro discussed ongoing trials in prevention and treatment of type 1 diabetes, respectively.

PATHOPHYSIOLOGY

Type I diabetes is an autoimmune disorder. Both genetics and environmental components play a role in the pathogenesis of the disease. The emerging hypothesis about the pathophysiology of type 1 diabetes is that a heterogeneous genetic component and one or more autoantigens interact to trigger the autoimmune process. Ultimately, type 1 diabetes represents a heterogeneous and polygenic disorder, with a few rare examples of monogenic mutations leading to autoimmune diabetes.

Early studies in identical twins who develop type I diabetes indicate the heterogeneous nature of this disorder. If the first twin develops type I diabetes after the age of 25 years, then the risk of subsequent development of diabetes in the other twin is less than 5%. However, if the first twin develops type I diabetes before the age of 5 years, the risk for the second twin is very high, approximately 50% to 60%. These data suggest the polygenic nature of this disease and the general difficulty in assessing risk.

In the general population, HLA alleles play a predominant role in determining the risk of type I diabetes. If individuals have the same DR and DQ haplotype, they have a very similar risk for type I diabetes no matter where they live. The frequency varies tremendously in populations; interestingly, some alleles that convey dominant protection are observed.

Specific genetic mutations or polymorphisms have been identified that convey the risk of type 1 diabetes. The MHC gene polymorphisms are the clearest indicators of risk for disease. Recently, a polymorphism of the insulin gene has been identified that suggests it influences risk for type I diabetes. This polymorphism influences the expression of insulin messenger RNA within the thymus and many of the lymphoid organs and may influence immune function by peripheral antigen-expressing cells. Clear examples of monogenic lesions or mutations that lead to type I diabetes also exist. The best recognized is the autoimmune polyendocrine syndrome type I. In this syndrome, a mutation on chromosome 21 is present that leads to type 1 diabetes. Recently, an additional monogenic mutation was identified and referred to as X-linked polyendocrinopathy, immune deficiency, and diarrhea syndrome. Others have reported on polymorphisms in the interleukin-12 gene that also convey risk. In summary, common polymorphisms exist in the population that are associated with diabetes, as well as a few well-defined monogenic mutations, and these may be useful genetic predictors of risk. However, many of these genetic studies need to be verified in diverse populations. Recent advances in genetics and genomics should greatly help identify the genes involved in type 1 diabetes.

IMMUNE TRIGGERS

Many antigens have been proposed to be the autoantigens that trigger the autoimmune process in diabetes.

From the Research Department, Juvenile Diabetes Research Foundation International, New York, NY.

This symposium was sponsored by the American Federation for Medical Research and the Juvenile Diabetes Research Foundation International.

Address correspondence to: Richard Furlanetto, MD, Juvenile Diabetes Research Foundation International, 120 Wall Street, New York, NY 10005-4001. E-mail rfurlanetto@jdrf.org

These include insulin peptides, GAD65, IA-2 and others. Insulin is expressed in the thymus, the lymph node, and the spleen, in a subset of dendritic cells and macrophages. Autoantibodies to these molecules have been observed in at-risk individuals as well as in patients with new onset type 1 diabetes. Additionally, the best-studied autoimmune diabetes animal model, the NOD mouse, has insulin autoantibodies, but not GAD antibodies or IA-2 autoantibodies. More recently, evidence suggests that the B9–23 peptide of the insulin molecule may be the autoantigen in type 1 diabetes. T cells reacting with the B9–23 peptide of insulin in the NOD mouse can transfer diabetes. In humans, the B9–23 peptide binds very well to the human DQ-8 molecule.

Administration of the B9–23 peptide of insulin to NOD mice usually produces autoantibodies in 4 to 8 weeks, and a subset of the mice then go on to develop diabetes. Of T-cell clones isolated directly from the islets of NOD mice, 50% recognize insulin and 97% recognize the B9–23 peptide. These T-cell clones can transfer diabetes rapidly into young NOD mice. Additionally, the B9–23 peptide itself, like many other molecules, can prevent the development of diabetes in the majority of NOD mice. The antibody response to the B9–23 peptide has a typical MHC-restricted response. Data suggest that B lymphocytes in a normal mouse are not tolerant to insulin and that T lymphocytes in a normal mouse are not tolerant to the B9–23 peptide. The data further suggest that endogenous insulin or proinsulin is processed to a B9–23-like peptide or that a possible environmental mimitope of B9–23 exists.

In summary, antigens are likely required to trigger the autoimmune process in type 1 diabetes. Numerous possible antigens have been identified. However, whether the antigens come endogenously, from within the individual, or from an environmental exposure or mimitope remains to be determined. Furthermore, which antigen is the primary antigen or whether multiple antigens are responsible for trigger of the autoimmune disease has yet to be conclusively defined.

IMMUNE TOLERANCE

The induction of immune tolerance has been recognized as achievable for about 1700 years, but researchers today are trying to learn how to do it consistently and reproducibly. Immune tolerance is a selective lack of an immune response to targeted antigens. Immune tolerance is important for type 1 diabetes when one considers therapeutic approaches such as islet transplantation, in which an allogeneic immune response is generated. Also, immune tolerance may play an important role in preventing disease onset or disease progression in this autoimmune disorder. Islet transplantation, ie, replacing the cells destroyed by the immune system in patients with type 1 diabetes, perhaps offers the best hope of restoring normal metabolism in type 1 diabetes. The primary problem is that toxic life-long immune suppression, required in organ transplantation, would not be desirable in those patients who are well controlled with exogenous insulin. Therefore, protocols that induce immune tolerance are essential for furthering cell-replacement therapies as well as preventative measures for type 1 diabetes.

Through 1999, success with allogeneic islet transplantation was minimal and required extensive immunosuppression. These mostly unsuccessful transplantations used standard immunosuppressive regimens, including cyclosporine, corticosteroids, and other drugs. Recently, Dr Shapiro and his team in Edmonton reported on a modified immunosuppressive protocol that was steroid-free and used sirolimus (rapamycin), tacrolimus, and daclizumab (monoclonal antibody against the interleukin-2 receptor). This combination produced more successful results, likely because the protocol uses no steroids and needs an abundant amount of islets, usually requiring multiple organ donors.

It will be essential to eliminate life-long immunosuppressive protocols, which increase the risk for development of lymphomas, cancers, or serious infections, are usually detrimental to the beta cell itself, and increase insulin resistance. A functional definition of allograft tolerance is “survival of transplanted tissue in normal recipients in the absence of chronic immunosuppression, either with a transient treatment or no treatment.” A number of anecdotal cases with kidney allografts suggest that long-term immune tolerance is possible. What remains to be determined is how to do this predictably and reproducibly in islet transplantation.

Allogeneic tolerance can be achieved by blocking T-cell activation. Three basic steps in the T-cell activation process are as follows: 1) the T cell recognizes the autoantigen and becomes activated; (2) the activated T cell upregulates a costimulatory molecule called CD40 ligand (or CD154), which engages the CD40 molecule on the antigen-presenting cells; within 24 to 48 hours, this interaction leads to the upregulation of B7 (or CD80), which 3) interacts with CD28 on the T cell and induces secretion of a number of cytokines, which also activate the T cell.

These three major steps represent targets for blocking T-cell activation and inducing immune tolerance. One could attempt to block T-cell recognition of antigen or block the interaction between CD40 and the CD40 ligand. Also, one could try to block the last step in the process, the costimulatory step, in which the B7 and CD28 molecules

interact. In the clinic, several molecules are being tested to block T-cell activation, including anti-B71 antibodies and CTLA4-immunoglobulin. Additionally, an anti-CD154 antibody is being tested to block the second step in activation. In animal studies, anti-CD154 antibody has been shown to induce tolerance in 90% to 100% of islet allograft recipients. However, this approach has not worked with islet transplantation in an autoimmune background, namely in the NOD mouse. Combination approaches that block many steps of T-cell activation process will need to be examined. Investigators will need to focus on the situation with primed allo- or autoreactive T cells and how a costimulation blockade can be used to overcome a primed response, not just a naive response.

PREVENTION

Despite public perception that insulin is a cure for diabetes, this treatment is not making a big enough impact on the disease's morbidity and mortality. In the past 20 years, deaths resulting from diabetes have continued to increase, whereas morbidity and mortality from cancers, cardiovascular disease, and stroke have decreased. This creates a sense of urgency to prevent and cure diabetes. Preventing diabetes could involve identifying the genes that cause the disease or the environmental agents that trigger the autoimmune process. Knowledge of the environmental trigger might make it possible to remove the offending agent or prevent exposure in susceptible individuals. However, identifying the trigger remains elusive.

For decades, there has been speculation that the trigger is a virus, such as one of the Coxsackie B viruses. Although there is some anecdotal and epidemiological evidence supporting this, there is no solid evidence that unequivocally associates any virus with diabetes. A number of dietary agents also have been proposed as triggers, including cow's milk or breast-feeding. Again, if we knew the roles of those involved, we might be able to remove them. Another way of finding a prevention and cure would be to take a genetic approach. If diabetes were a single-gene-defect disorder, it might lead to some therapeutic avenues, but in most people, it is likely that a number of genes play a role in the onset of the disease. Because of difficulties in identifying precise genes or environmental agents involved, the focus of most prevention studies has been modulating the immune system.

In the NOD mouse, nearly 150 ways have been identified to prevent diabetes. However, few of these molecules could induce remission after disease onset. Of the 150 or so agents that prevent diabetes in the NOD mouse, only a handful of agents are actually undergoing human clinical trials. For example, Kevan Herold at Columbia University is leading a multicenter trial with the anti-CD3 antibody. Additional large prevention trials are underway:

- The first, funded by National Institutes of Health, the JDRF, and the American Diabetes Association, is called the DPT-1, or Type I Diabetes Prevention Trial and has been ongoing since 1994. It is a secondary disease, multicenter, randomized, double-blinded, and placebo-controlled prevention trial that will screen close to 100,000 individuals by the time it is completed. It seeks to screen relatives of people with type I diabetes and to find 340 patients who were considered at high risk as well as 490 patients considered at intermediate risk. The relatives are screened for the presence of islet cell antibodies or the cytoplasmic form of ICA. Individuals were put into two different groups. Those considered at high risk for diabetes were put on an insulin injection protocol involving two subcutaneous injections per day, with once-a-year intense insulin therapy. Those who had an intermediate risk were given a placebo. The first results of this trial are pending.
- Another major trial is the European Nicotinamide Diabetes Intervention trial, which is similar to the DPT-1. It was a secondary disease prevention trial that involved the screening of relatives who had an ICA of greater than 20 JDF units. It started in 1994, ended in 1998, and screened 40,000 relatives to find individuals with the antibody; 552 participants enrolled. Again, the goal was to learn whether nicotinamide, whose activities are listed here, could actually prevent the onset of diabetes. The investigators hope to report the results in April 2003.
- The third large diabetes prevention trial is the Trial to Reduce IDDM in the Genetically at Risk. This one differs from the other two in that it is a primary disease prevention trial. Its hypothesis is that exposure to cow milk in infancy is the major factor involved with promoting diabetes. The double-blinded trial is predominantly being conducted in Finland and Canada but involves other countries and includes a few centers in the United States. They hope to include more than 5000 newborns in this study, for which they will monitor exposure to cow milk and breast-feeding practices.

In summary, a few molecules shown to prevent disease in animals are being tested in clinical studies in people. Additional trials are being launched. The National Institute of Diabetes, Digestion & Kidney Disease has established a new clinical trial network called Trial Net. Trial Net will establish programs throughout the country in which scientists can propose what form of therapy they think would be effective in preventing diabetes. Essentially, diabetes investigators are taking a step backward to smaller pilot

trials to take the major step forward to identify what is the next agent that should be used nationally to prevent diabetes. This is important because there are a number of agents, some familiar, such as the B9–23 peptide, and others less well-known, that are candidates for testing in prevention trials.

TREATMENT

Replacing the lost beta cells by islet transplantation is one possible treatment for type 1 diabetes. Until 1998, only 8% of people receiving islet transplants had gained insulin independence for a year. Many of these earlier protocols relied on standard immune suppression regimens such as cyclosporine and steroids. The Edmonton Protocol, which we developed in Alberta over the past several years for furthering islet transplantation, uses a corticosteroids-free regiment and greater islet mass (from two or more donor pancreata if necessary). The aim was to use drugs that suppressed the immune response without contributing to diabetes. In detail, the protocol includes islet-alone transplantation in patients with type I diabetes who have had long-standing diabetes, who are C peptide–negative, and who have the complications of reduced awareness of hypoglycemia, metabolic lability, and uncontrolled diabetes despite compliance with an insulin regimen. We transplanted islets that were approximately 70% to 80% pure. The packed cell volume is approximately 3 to 4 mL in each transplant. Some patients required two transplants to achieve the total of 700,000 islets, or greater than 10,000 islet equivalents per kilogram.

Fifteen patients had undergone transplantation with this protocol at the time of this writing. Ten of our 15 patients who underwent transplantation had islets derived from two donors. Four of the heavier patients required islet cells from three donor pancreata. The estimated average islet mass transplanted was approximately 12,000 islet equivalents per kilogram. The transplantation procedure itself is very straightforward. The cells are drawn up into a syringe and infused into the portal vein of the liver via a 5-French Kumpe catheter. The portal pressure in the portal vein is measured before, during, and after the islet infusion. When the packed cell volume is approximately 3 to 4 mL, very little change in the portal pressure is observed. The catheter is withdrawn and the patient remains on his or her side for around 4 hours, and then is usually discharged within 12 to 24 hours.

The demographics for our 15 patients include a median age of 40 years; all had long-standing diabetes of median duration of 31 years. All these patients were C peptide–negative, and they had the secondary complications of diabetes, excluding endstage renal disease. The predominant indication was severe metabolic lability. All 15 patients were able to achieve insulin independence for variable periods of time. The median follow-up for the first seven patients was 22 months. All patients had complete resolution of their hypoglycemia reactions, and as a consequence of that, had a marked improvement in their overall quality of life at that time. All fifteen patients had detectable C peptide, but there were three more recent patients who required exogenous insulin to maintain normal blood glucose concentrations. One of these patients seemed to have developed insulin-resistant type 2 diabetes after islet transplantation. We believe tacrolimus may add to this insulin resistance. The second patient lost some partial function of the graft as a result of a peripheral portal vein branch thrombosis, and the third patient lost some partial function possibly because of graft rejection. In summary, three of 15 patients used approximately one fifth of their pretransplantation insulin dose, and all had positive C peptide.

The hemoglobin A1Cs were normalized or near normalized after islet transplantation and remained so in long-term follow-up of the first seven patients. The hemoglobin A1C results were more normalized in our islet transplant recipients than those reported for insulin injection in the DCCT trial. This suggests the patients will likely avoid or decrease the chance of complications of diabetes. In our islet cases, we had seven to 15 mismatches, but we did not transmit a single case of cytomegalovirus. Transmission of cytomegalovirus is common in whole organ transplant programs.

Additionally, at the time this article went to press, the first seven patients did not seem to have been sensitized to their donors, even though they had more than one donor.

None of the patients had developed lymphomas, cancers, or serious infections by this point. Of course, long-term follow-up is required as these are potential risks, given the potent immunosuppression that these patients received. Only minor complications were observed in the initial series of transplantations, including non–life-threatening bleeds that were resolved by reducing the dose of heparin to 35 U/kg. Additionally, three patients had transient elevations in liver function tests shortly after the islet infusion, and these resolved entirely with time. The vast majority of patients had no change in renal function over time, but there were two patients with elevated baseline serum creatinine. These two patients had significant elevation in their creatinine after transplantation. This suggests the low-dose tacrolimus-sirolimus combination could be nephrotoxic in patients with inadequate baseline renal reserve. In these two patients, we later observed completely withdrawn tacrolimus. We initiated treatment with CellCept and observed some improvement in their

renal function. In future trials, we plan to screen patients closely and, in those who have elevated baseline creatinine, to use only the combination of sirolimus-CellCept and not to use tacrolimus.

To bring islet transplantation to a widely used therapy for type 1 diabetes, many hurdles remain. Other institutions and hospitals must be able to isolate islets and transplant. Future plans include a 10-center trial to reproduce the Edmonton Protocol in 40 patients. Many other programs that are now intending to move forward with islet transplantation worldwide have been visiting those and other centers. There are now seven sites identified in the United Kingdom, six centers as a consortium in France, and six other European centers, as well as possibly up to 20 other US sites. Additionally, life-long immune suppression must be overcome, perhaps with the development of tolerance protocols. Finally, there is a fairly steep dose-response curve requiring 9000 to 10,000 islet equivalents per kilogram to achieve insulin independence in the majority of patients. Potential approaches to achieve shifting the dose-response curve to the left are using new drugs, particularly those that do not involve any calcineurin inhibitors, and therefore might allow better function of grafted cells. Promising treatments include the use of anti-inflammatory strategies, perhaps as simple as aspirin, or anti-TNF alpha soluble complement receptor-1 antibodies; modification of the islets ex vivo with anti-apoptotic pathways to help protect and make those islets more robust; and better mechanical methods to improve the isolation efficiency. Finally, the ultimate challenge of finding a new and sufficient source of islet remains. Potential new human islet sources include human embryonic stem cells or induction of growth of adult stem cells, either derived from the ductile elements or from within islets. The ultimate goal in implementing islet transplantation as a cure for type 1 diabetes is transplantation without immune suppression.

ACKNOWLEDGMENTS

The Juvenile Diabetes Research Foundation International would like to thank the speakers: George Eisenbarth, MD, PhD, of the Barbara Davis Center for Childhood Diabetes; Dale Greiner, PhD, of the University of Massachusetts Medical School; Mark Atkinson, PhD, of the University of Florida; and A. James Shapiro, MBBS, FRCSC, of the University of Alberta.