Immunologic Issues in Type 1 Diabetes

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This is the second in a series of reports on the American Diabetes Association (ADA) 61st Scientific Sessions held in Philadelphia, PA, in June 2001. It covers topics related to immunity and type 1 diabetes.

Jay Skyler, Miami, FL, presented the results of the parenteral substudy arm of the Diabetes Prevention Trial for Type 1 Diabetes (DPT-1), in which relatives of patients with type 1 diabetes, whose risk of type 1 diabetes is 10- to 20-fold greater than that of the general population, were screened for islet cell antibodies (ICAs) and enrolled in a study of insulin administration if they showed high risk of developing diabetes. Animal studies in female nonobese diabetic (NOD) mice have shown this to be an effective treatment, and in the early 1990s, Keller et al. (1) reported a pilot study of 12 individuals with a predicted risk of diabetes; 5 subjects were treated with insulin and 7 subjects declined treatment. By 2.5 years, all of those who declined treatment had developed diabetes; at 5 years, only half of the intervention group had the disease.

The DPT-1 was initiated in 1993. Screening began in February 1994 and randomization began in January 1995; 339 subjects were enrolled, with follow-up ending 13 April 2001. Screening of 89,827 relatives, with 84,228 samples analyzed, showed that 3,152 (3.7%) were ICA positive. A total of 2,103 subjects were staged, 535 having low first-phase insulin response. Of 372 subjects who were eligible for randomization, 339 were randomized. Skyler pointed out that 354 of the 3,152 ICA-positive individuals had actually already developed diabetes, with 156 identified before further testing. The projected annual event rate was 21%, and the actual finding was of 15.1% developing diabetes (in addition to those developing diabetes before randomization). Of the patients who developed diabetes, 60% were sibs, 25% were parents, 4% were offspring, and 8% were other relatives. All were ICA and insulin autoantibody (IAA) positive. At baseline, 48.1% were female, 45.4% were below age 10 years, 38.9% were 11–20 years of age, and 15.6% were over 21 years of age; 93.5% were Caucasian, reflecting the ethnic distribution of type 1 diabetes in the U.S., although ICA positivity was seen in ~3% of relatives, regardless of ethnic background.

A total of 169 were in the intervention group, and 170 were observed. Individuals were followed for an average of 3.7 years. Of the subjects followed, 60% developed diabetes by 5 years. Those with impaired glucose tolerance (IGT) and those with normal glucose tolerance had a 22 and 10% rate of diabetes development per year, respectively. Those <6 years of age progressed at a rate similar to those aged 6–12 years. Those >15 years of age progressed at a much slower rate, which implies that studies confined to older individuals will need even larger numbers. Those with ICA alone progressed at a slower rate than those with the presence of more than one antibody.

Coming as a great disappointment to the audience, Skyler reported, "There was no impact of [parenteral] insulin therapy on delaying or preventing the onset of diabetes." Also, and again disappointingly, C-peptide showed no difference with or without treatment. The insulin intervention did cause some hypoglycemia. There were 93.6 presumed events (not documented but responding to carbohydrate ingestion) per 100 patient years in the treated patients, as opposed to 57 events in the control subjects. Documented hypoglycemia occurred in 59 vs. 11 patients/100 patient-years, and severe hypoglycemia did not occur. There was no difference between the groups in any measure of cognitive function, and those with definite hypoglycemia also had no change in cognitive function. Skyler suggested that, perhaps, this group was studied "too late." An ongoing oral antigen trial in subjects with a 5-year risk of 26–50% (less than that in the parenteral arm) is in progress and may be successful, despite the current study being negative.

Stoever et al. (1092-P) reported that the dose of subcutaneous insulin administered in the DPT-1 did not suppress endogenous insulin secretion, suggesting that failure of the study to prevent diabetes may be related to lack of β-cell rest (abstract numbers refer to the Abstracts of the 61st Annual Meeting of the American Diabetes Association, *Diabetes* 50 [Suppl. 2]:1–A649). Insulin treatment in the study may, however, have suppressed the T-cell response to human islet proteins. Brooks-Worrell et al. (313-PP) studied a group of 16 patients in the DPT-1, showing that there was some immune effect of the intervention. T-cell responses to human islet proteins, measured using cellular immunoblotting, showed that five of six untreated patients showed response to a mean of eight islet antigens, while three of the insulin-treated patients showed no response and, on average, there was response to only one antigen, similar to that seen in individuals from a normal population. At follow-up, the untreated patients showed increasing response, while none of the 10 treated patients did so. Thus, insulin treatment may produce an immunosuppressive effect on T-cell proliferative responsiveness to islet proteins in subjects at high risk for developing clinical type 1 diabetes, although no clinical benefit was demonstrated.

Several studies at the ADA meeting did show hints of promising treatment strategies. Vitamin E given with nicotinic acid in a setting of tight glycemic control

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Abbreviations: ADA, American Diabetes Association; DCCT, Diabetes Control and Complications Trial; DPT-1, Diabetes Prevention Trial for Type 1 Diabetes; GADA, antibody to GAD; IA2, insulinoma-associated antibody; IAA, insulin autoantibody; ICA, islet cell antibody; IGT, impaired glucose tolerance; IL, interleukin; iNOS, inducible nitric oxide synthase; IV, intravenous; LADA, latent autoimmune diabetes of the adult; NO, nitric oxide; NF-κB, nuclear factor-κB; RAGE, receptor for AGE; STZ, streptozotocin; TNF, tumor necrosis factor.

may lead to prevention of β-cell loss in patients with new onset type 1 diabetes. Pozzilli et al. (287-PP) treated 56 patients with new onset type 1 diabetes (mean age 7.8 years) with intensive insulin and with 25 mg · kg^{-1} · day^{-1} nicotinic acid with or without 15 mg · kg^{-1} · day^{-1} vitamin E in a randomized controlled study. The addition of vitamin E reduced the insulin dose requirement and HbA_{1c} and led to a C-peptide peak at 6 months of 0.38 vs. 0.27 nmol/l. Åkerblom et al. (17-LB) described the results of the second pilot study of the Finnish Trial to Prevent Diabetes in the Genetically At-Risk (TRIGR), with 208 newborn infants who had a first degree relative(s) with type 1 diabetes with the risk-associated HLA-DQB1 alleles (*02 and/or *0302) in the absence of protective alleles. After breast-feeding, the infants were randomized to either a casein hydrolysate or a conventional cow's milk–based formula until the age of 6–8 months. Seroconversion to positive ICA was decreased by 67% and to positive IAA by 62%, with a 66% decrease in the likelihood of developing at least one positive antibody in the group fed the casein hydrolysate. To show a decrease in the rate of development of type 1 diabetes will require a large-scale clinical trial. In a fascinating animal study, Arany et al. (333-PP) reported an approach to preventing diabetes in the NOD mouse. The insulitis in this model is preceded by a decrease in β-cell mass, and it is possible to increase β-cell mass by supplementation of the mother during fetal development. Dietary taurine supplementation of the mothers was found to increase β-cell mass, to decrease the histologic findings of insulitis, and to delay the onset of diabetes in the offspring. Whether this will prove applicable to human diabetes is uncertain.

A number of studies reported at the meeting gave additional information pertaining to the nature of the autoimmune defect in type 1 diabetes. Hummel et al. (314-PP) studied 1,920 offspring of patients with type 1 diabetes, with 1.1% of 1,514 having positive antibodies at 9 months, 3.2% of 1,154 positive at 2 years, and 7.3% of 550 positive at 5 years. The cumulative risk of diabetes was 3.2% from fathers and 2.0% from mothers at 5 years, a pattern that has been previously reported. Patterns of antibodies changed and the number of positive antibodies decreased with later onset, suggesting that those with earlier positive antibodies were at higher risk. Hathout et al. (336-PP) reported findings in 24 patients developing type 1 diabetes before age 5 years, at an average age of 2.6 years. Of these patients, 17% had positive ICA and 21% had positive GAD antibody, which is less than that seen in older children, but the prevalence of the high-risk HLA-DQA1 alleles *0501 and *0301 were 58.3 and 54.2%, rates higher than those in older children, suggesting differences in this young-onset group. Kukreja et al. (315-PP) reported a deficiency of natural killer T-cells in both patients with newly diagnosed and longstanding type 1 diabetes and in relatives with multiple positive autoantibodies, suggesting another immunologic hint as to pathogenesis. In mice and humans, the presence of reduced numbers of these cells has been associated with the development of autoimmune diseases. Kukreja also reported that the cells showed decreased secretion of interleukin (IL)-4 and γ-interferon after stimulation. Diez et al. (18-LB) studied the phenomenon related to self-tolerance of "self–antigen-presenting cells" present in both thymus and peripheral lymphoid organs, expressing autoantigens such as insulin, GAD, and tyrosine phosphatases (IA-2). Using a monoclonal antibody against proinsulin, they showed that such cells with a surface-staining pattern of proinsulin peptides were present in lymphoid organs and blood. Using flow cytometry analysis, they were able to show by use of two antibodies that cells expressing proinsulin were present in the spleen and peripheral blood. The cells expressed insulin mRNA and markers of antigen-presenting cells, such as CD11c, CD14, CD40, and HLA-DR, suggesting an important additional component of the prevention of islet cell autoimmunity.

C-peptide

C-peptide deficiency has been suggested to play a role in the complications of diabetes. A C-peptide receptor has been identified in the kidney, where it appears to function to reduce glomerular blood flow. Consistent with these findings, administration of C-peptide to type 1 diabetic patients has been observed to reduce the urinary albumin excretion. Fiorina et al. (343-PP) studied 16 patients with type 1 diabetes who had had combined islet and whole kidney transplants 4 years earlier. All of the patients had adequate renal function; nine had and seven did not have some degree of islet function. Although HbA_{1c} was not significantly lower, C-peptide was seven time higher, erythrocyte Na-K ATPase was 30% higher, and urine albumin decreased from 109 to 85 mg/24 h versus an increase from 92 to 184 mg/24 h from the initial level after transplantation. The same group (618-P) compared nine patients with versus seven patients without C-peptide >0.5 ng/ml at least 1 year after kidney plus islet transplant, with similar HbA_{1c}, creatinine, lipid, and blood pressure levels. Endothelium-dependent brachial artery dilation was 7.8 vs. 0.5%, basal nitric oxide (NO) production more than doubled, and carotid intima-media thickness was unchanged compared with an increase of 0.12 mm in the two groups, suggesting preservation of vascular function. In patients with type 2 diabetes, Saito et al. (730-P) reported C-peptide levels of 3.1 vs. 4.8 ng/ml in 15 with vs. 52 patients without microalbuminuria. Johansson et al. (1052-P) reported that infusion of C-peptide (5 pmol · kg^{-1} · min^{-1} for 60 min) to 8 men with type 1 diabetes improved systolic and diastolic myocardial function measured by pulsed tissue Doppler before and during dipyridamole stress. Bjork et al. (358-P) treated 56 children aged 7–17 years at onset of type 1 diabetes with either the K^+ channel opener diazoxide to inhibit insulin release or with placebo for 3 months. Stimulated C-peptide levels were higher after treatment through 12 months, though similar to the control group at 24 months, suggesting the potential for preservation of endogenous insulin and C-peptide secretory function. A number of animal studies showed effects of C-peptide on nerve function. Cotter and Cameron (748-P) reported that C-peptide administration to streptozotocin (STZ)-diabetic rats caused 62 and 78% improvement in sciatic motor and saphenous sensory nerve conduction velocity, respectively, which was prevented by coadministration of the NO synthase inhibitor L-nitro-arginine. Sima et al. (242-OR) reported that C-peptide administration prevented the upregulation of the low-affinity insulin receptor seen in sciatic nerves in an animal model of type 1, but not type 2, diabetes. Sima et al. (770-P) administered human C-peptide in doses of 10, 100, 500, and 1,000 μg · kg^{-1} · day^{-1} for 2 months in a rat type 1 diabetes model, showing a dose response in increasing nerve conduction

velocity and Na^+K^+-ATPase activity and in preventing histological changes.

C-peptide and islet antibodies in diabetes management

Jerry Palmer, Seattle, WA, discussed the utilization of C-peptide in the treatment and management of type 1 diabetes. Because every insulin molecule is produced along with a C-peptide molecule, the measurement of C-peptide allows assessment of insulin secretion in insulin-treated patients, many of whom develop anti-insulin antibodies. Antibodies to C-peptide may, however, recognize proinsulin or proinsulin split products, leading to differences between findings of different studies. The limit of detection of most clinical assays is 0.1 pmol/ml. In pancreatectomized patients, glucagon-stimulated C-peptide is at the detection limit of the assay. However, patients with type 1 diabetes with sufficient endogenous insulin to improve glycemic control have levels of 0.2 pmol/ml, non-insulin requiring individuals may have levels of 0.6 pmol/ml, and normal levels may be at 1.0 pmol/ml; thus, small differences are meaningful. Palmer favored the use of stimulated C-peptide, though noted excellent correlation with fasting values, as some patients with very low fasting levels show responses to stimulation and some with higher fasting levels have flat responses. Measuring both fasting and stimulated levels is ideal. Comparing the response to intravenous (IV) glucagon, IV glucose, a mixed meal, and oral glucose, he noted that individuals with type 1 diabetes have little response to oral glucose and respond best to a mixed meal or IV glucose, with glucagon having the disadvantage of causing gastrointestinal side effects such as nausea. Reproducibility is a problem, with glucagon-stimulated C-peptide having 15% intrasubject variation in normal control subjects and greater variation in patients with diabetes, particularly as the β-cell lesion changes over time, and they may have different basal glucose levels at different times of testing. One can use C-peptide and C-peptide clearance measurements to assess secretion of insulin, which is extracted to a variable degree by the liver.

C-peptide measurement is useful in distinguishing type 1 from type 2 diabetes, although this has become complicated in both children and adults who have both obesity and type 1 diabetes. Obesity increases insulin resistance, but does not protect against autoimmunity, and may result in type 1 diabetes presenting at a time when there is a greater degree of β-cell function and, hence, higher C-peptide levels. Antibody-positive patients with type 2 diabetes show C-peptide levels similar to those in antibody-negative patients at diagnosis. Over several years, their C-peptide levels decrease more than those of antibody-negative patients. Stimulated C-peptide levels of 0.6 pmol/ml are useful in distinguishing between those with type 2 diabetes who do and do not require insulin, the former showing great overlap with type 1 diabetes in C-peptide levels.

C-peptide levels are related to the ability of type 1 diabetic patients to attain good glycemic control. In the Diabetes Control and Complications Trial (DCCT), from which patients with C-peptide levels >0.5 pmol/ml were excluded, 552 of the 855 subjects with diabetes for 1–5 years had standardized meal-stimulated levels <0.2 pmol/ml, and 303 had levels between 0.2 and 0.5 pmol/ml. Before study entry, the latter had HbA_{1c} levels 1% lower than nonresponders, and this difference was maintained for 7 years in the DCCT. The C-peptide–positive group experienced less hypoglycemia, and other studies have shown an increased glucagon response to hypoglycemia for these patients. Comparing patients with C-peptide <0.1, 0.1–0.2, and >0.2 pmol/ml, the latter showed lower fasting glucose and HbA_{1c} levels, and the insulin dose requirement was somewhat lower in the 0.1–0.2 pmol/ml group. It is not uncommon for patients with type 1 diabetes to "have amounts [. . .] that make any difference," Palmer noted, with almost half of the 4,000 patients screened for the DCCT having stimulated C-peptide >0.2 pmol/ml between 2 and 5 years after diagnosis. In the future, use of C-peptide for monitoring efficacy of treatments to preserve β-cell function may be useful. Palmer pointed out that the assay is well standardized and that commercial C-peptide assays are usually reliable. Other uses of C-peptide may include the assessment of need for insulin in patients with type 2 diabetes who were transferred to insulin because of sulfonylurea failure.

Palmer answered a series of questions illustrating additional uses of C-peptide. As far as the measurement of fasting rather than stimulated C-peptide, he noted that their correlation shows an *r* value of ~0.8. A fasting C-peptide concentration of 0.2 corresponds to a stimulated C-peptide level of 0.6 pmol/ml, but "the noise around 0.2 is a lot greater." Ideally, he said, C-peptide should be measured with a basal glucose of ~120 mg/dl, as higher levels may stimulate β-cells or, less typically, decrease C-peptide levels because of glucose toxicity. For stimulation, in addition to standardized mixed meals, one "can use a variety of nonglucose stimuli," including isoproterenol, arginine, and GLP-1. He commented that the measurement may be useful "whenever the patient's diabetes is behaving unusually." As far as latent autoimmune diabetes of the adult (LADA), "type 1½ diabetes," and other difficult-to-characterize forms, he suggested, "The classification system is in evolution. In the future, we will rely a bit more on immune markers." As far as the use of C-peptide to decide on insulin treatment for patients with type 2 diabetes, "most of the data come from the time when all we had was sulfonylureas." Patients who failed to respond to sulfonylureas and are receiving insulin treatment but have stimulated C-peptide >0.6 pmol/ml could be given a trial of oral agents. As far as the guidelines that C-peptide be used for determination of whether a specific patient be treated with insulin pumps, he noted that there is no evidence that this helps to determine who will and who will not benefit from insulin pump treatment. If the rationale is to distinguish between type 1 and type 2 diabetes, it would be more useful to measure antibodies.

William Winter, Gainesville, FL, discussed islet autoantibody markers in the diagnosis, prediction, and clinical course of autoimmune type 1 diabetes. He recalled the 1997 ADA recommendation that diabetes be divided into type 1, type 2, gestational, and a group of miscellaneous types, with type 1 diabetes divided into an autoimmune type (1A) and one with insulinopenia in the absence of islet autoantibodies (1B). Autoantibodies can be important in disease management, perhaps lessening the likelihood that an individual will develop ketoacidosis at onset of type 1 diabetes, or in determining whether a person with diabetes and obesity causing insulin resistance actually has type 1 or type 2 diabetes. Autoantibodies may play a role in determining prognosis

for a patient or in assessing the risk for other family members.

The four major antibodies measured clinically, ICAs, IAAs, GAD antibodies (GADAs), and insulinoma-associated antibodies (IA2s), are just a subset of the many different ones that have been described. It is unlikely that any are pathogenic. ICA was first described over 25 years ago, requiring measurement by indirect fluorescence, which is labor intensive, requires intensive quality assurance efforts, and can best be performed with human pancreas specimens. Histologically, these polyclonal IgG antibodies react with all cells of the islet, recognizing a variety of antigens. At diagnosis, ICAs are present in 70–80% of Caucasians and in fewer African-Americans, with decreasing prevalence over time. ICAs are present in 0.4% of nondiabetic individuals and therefore show high specificity. ICAs are present in 2–3% of first-degree relatives and do not appear to occur in pediatric type 2 diabetes. They are found in LADA.

IAAs are the only β-cell–specific autoantigens. Measurement must be performed before starting insulin treatment, as the assay cannot distinguish between induced antibodies and autoantibodies. GAD, the "64K autoantigen," converts glutamate to the neurotransmitter γ-amino-butyric acid and is ubiquitous in the central nervous system. Type 1 diabetes is associated with specific epitopes of GADAs. These are present at similar frequency to ICA at diagnosis. GADA persists after diagnosis, making it useful for patient assessment several years after disease onset. IA2 is a member of the protein tyrosine phosphatase family, a group of receptor and cytoplasmic signal transduction enzymes present in many cell types. ICA 512 recognizes the cytoplasmic portion of this antigen. IA2 is present in 60% of patients at the time of onset of type 1 diabetes. However, both GADA and IA2 are found in 2–3% of the normal population, making their specificity lower than that of ICA.

Winter noted that fewer than 15% of individuals with type 1 diabetes have an affected first-degree relative. A patient with type 1 diabetes has a 5% chance of a sibling being affected, and concordance in identical twins is 30–50%. Thus, environmental factors must play a role in disease onset. An example of this is the puzzling finding that the frequency of type 1 diabetes in offspring is higher if the father has type 1 diabetes than if the mother is affected. Antibodies provide the earliest markers of these environmental events, with islet autoantibodies being associated with the finding of insulitis on the cellular level. Subsequent clinically recognizable events are the abnormal first phase (1–3 min) insulin response to IV glucose, for which the individual needs to lose 50% of β-cell mass, and the development of a frankly abnormal oral glucose tolerance test, which usually leads to clinical diabetes within 2 years. The DPT-1 showed that with close observation, type 1 diabetes is asymptomatic in 70% of patients at the time of onset. Winter pointed out that this schema of the natural history of type 1 diabetes need not occur in all individuals, and antibody measurement may assist in the assessment of prognosis for a given individual.

In family studies, individuals with higher ICA titers have greater risk of developing diabetes, and ICA-negative individuals have no risk of disease. ICA-positive schoolchildren who do not have a relative with type 1 diabetes have a rate of progression to type 1 diabetes similar to that of first-degree relatives, and younger individuals with positive antibodies have higher risk. Combining islet cell autoantibody measurements and IV glucose tolerance testing, with antibodies present and a low first-phase insulin response, there is a >50% risk at 5 years regardless of antibody titer. The presence of IAA with ICA increases risk. GADA and IA2 also predict type 1 diabetes; GADA is associated with a 50% 5-year risk, with risk increasing at higher antibody titers, and with greater numbers of positive antibodies. Winter concluded that ICA has the greatest ability to predict risk but requires a methodologically difficult assay and that GADA and IA2 are the most usable for population screening. Asked about the validity of assays in commercial laboratories, Winter recommended ascertaining which kit is being used by a given laboratory and whether it has been correlated with results in a research setting. He pointed out that enzyme-linked immunosorbent assay (ELISA) is not as good as the radioimmunoprecipitation assay, and that the use of monkey rather than human pancreas for ICA measurements probably gives less sensitivity for ICA.

A final important question is whether, after diagnosis, the presence of antibodies is associated with differences in the course of type 1 diabetes. ICAs are not associated with different clinical presentation or with different clinical findings during the initial year after diagnosis, but may be associated with lower C-peptide levels at 2 years. IA2 and the presence of multiple autoantibodies are also associated with lower C-peptide levels at 18–24 months. This has not been shown for IAA, and different studies have shown GADA to be either protective or harmful, so that further research in this area will be important.

Asked whether the classification schema should be redefined based on antibodies as well as clinical findings, Winter commented that some patients initially appearing to have type 1B subsequently show antibody positivity, and that when many antibodies are tested, >95% are positive to at least one, so that it is uncertain whether "type 1B" actually represents a different disease. Why bother determining antibodies, then, if you are going to treat with insulin anyway? "I'm certainly not here to tell you," he stated, "that every child needs autoantibody testing." Winter suggested, however, that those with evidence of autoimmunity may then be at higher risk of autoimmune disease of the thyroid, of pernicious anemia, and perhaps of adrenal insufficiency, and that "if you recognize that somebody has type 1 diabetes, 5–15% of relatives will develop type 1." He acknowledged that "the results of the DPT-1 created a bigger problem for us," as prior data suggested that insulin treatment would help patients with positive antibodies, but with the negative results of the trial one cannot state that "insulin is really good for the β-cell" for such individuals, at least before onset of diabetes.

Immunology and type 1 diabetes

Tumor necrosis factor-α. In a symposium addressing mechanisms of development of type 1 diabetes, tumor necrosis factor (TNF), an inflammatory predictor of disease in type 1 diabetes, was highlighted. Icbald Grewal of Genentech, South San Francisco, CA, discussed the TNF supergene family and the role of TNF molecules and receptors in the pathogenesis of autoimmune diseases. Two ligands have been identified: one that is expressed in normal tissues at low levels and induces proliferation in certain tumors, and the other that is expressed in the immune system, T-cells, dendritic

cells, monocytes, and macrophages; is involved in B-cell stimulation; and causes lupus-like autoimmune diseases when overexpressed. There are two receptors for these ligands, with activation leading to nuclear factor-κB (NF-κB) expression. Administration of antibodies to block the ligands, or the use of fusion proteins of the Fc portion of IgG with portions of the receptor, blocks IgM production by B-cells and decreases splenic IgG1 production, with activity in autoimmune arthritis and neurologic disease models. A question being studied concerns their effect in experimental and clinical type 1 diabetes.

John Corbett, St. Louis, MO, discussed the effects of cytokines produced in islets on β-cell function and β-cell destruction. Each islet contains ~2,000 cells, of which <1% are macrophages. Macrophages activated by TNF-α release IL-1β, which binds to surface receptors on β-cells, leading to production of inflammatory signals such as NF-κB, which thereby leads to inducible NO synthase (INOS) activation in β-cells. NO production decreases mitochondrial function, leading to decreased ATP levels and a reduction in insulin secretion. Aminoguanidine, which decreases NO synthase activation, attenuates this effect. Thus, it appears that a cascade occurs where TNF-α signals macrophages, which in turn cause β-cells to produce cytokines causing β-cell damage by both apoptosis and, under stimulation by cytokines and NO, necrosis.

Richard Flavell, New Haven, CT, noted that autoimmune diseases occur when a repertoire exists of autoreactive cells capable of reacting with the tissue in question. Lymphocyte activation, perhaps because of viral infection or chronic inflammation, leads to tissue destruction when immunoregulatory mechanisms fail. TNF-α is an important signal for lymphocyte infiltration. The biologic consequence of diabetes depends on genetic predisposition. Neonatal, but not subsequent, TNF administration exacerbates the disease, leading to acceleration of β-cell apoptosis, dendritic cell infiltration, apoptotic fragments presenting via the dendritic cells to CD4 and CD8 T-cells, and the CD8 T-cells mediating β-cell destruction. The additional factor TNF-related activation-induced cytokine appears to slow expression of disease by increasing levels of protective CD25 T-cells, which blocks further differentiation of the cytotoxic CD8 cells.

Additional studies of immunology and type 1 diabetes

In a study presented at the meeting, Herold et al. (134-OR) administered splenocytes from already diabetic NOD mice, a model of autoimmune diabetes, to nondiabetic NOD recipients. Diabetes developed in 88 vs. 14% of mice pretreated with a soluble form of the receptor for AGE (RAGE), which binds ligands and prevents their access to the cell surface receptor. The islets did not show inflammatory infiltrate or express TNF-α or IL-1β . Blockade of RAGE/ligand interaction may provide islet protection against development of diabetes or recurrence after islet transplantation. The same investigators (138-OR) treated 12 individuals with type 1 diabetes for <6 weeks with non-FcR binding anti-CD3 monoclonal antibody. At 1 month, circulating lymphocytes decreased to 24% of basal levels, but fever and an eczematoid rash responding to antihistamines and nonsteroidal anti-inflammatory drugs developed in 9 and 10 patients, respectively. IL-10 levels increased, suggesting activation of Th2 cells. HbA_{1c} decreased from 9.4 to 6.2%, while falling from 8.3 to 7.7% in six control subjects, with meal-induced insulin secretion increasing in eight treated but only one untreated patient. Scholtz et al. (522-P) administered NBI-6024, an altered peptide ligand that has been shown to generate Th2-like cells that appear to block the autoreactive process in NOD mice, to 15 patients with type 1 diabetes, showing preliminary evidence of safety of this approach.

Krischer et al. (180-OR) administered oral insulin versus placebo for 1–3 years to 205 newly diagnosed antibody-positive type 1 diabetic patients, showing a trend to greater C-peptide secretory capacity with treatment. Braghi et al. (131-OR) reported that 9 of 36 patients receiving islet transplants showed an increase in antibodies to GAD consistent with reactivation of a quiescent autoimmune response. Contreras et al. (133-OR) administered Anti-F(Ab)2-immunotoxin, which ablates >99% of T-cells, with ~50% recovery by 5 months, and the NF-κB inhibitor deoxyspergualin, blocking proinflammatory cytokine release and dendritic cell maturation to seven rhesus monkeys with STZ-induced diabetes. After subsequent transplantation of islets from MHC class I and class II incompatible rhesus monkey donors, only two showed rejection, at 70 and 335 days, with the other five normoglycemic at 180, 386, 532, and 566 days. Genovese et al. (375-P) found GAD autoantibodies in 62 of 949 patients diagnosed as having diabetes after 40 years of age. Of antibody-positive patients, 61%—but 15% of those with negative antibodies—were treated with insulin, with HbA_{1c} 7.8 vs. 7.0% and BMI 26.2 vs. 29.1 kg/m^2, respectively, suggesting the importance of LADA in this population and the potential clinical benefit of GADA screening in adult-onset diabetes. In an interesting report on adverse effects of immunosuppressive treatment on the β-cell, Montori et al. (1710-P) reviewed 10 randomized trials, 11 cohort studies, and 1 case-control study of diabetes developing after transplantation of a variety of organs, which occurred at 1 year in 4–18% of patients without previous diabetes. Non-Caucasian race was associated with a 4.6-fold increased risk, and tacrolimus treatment was associated with a 2.3-fold increased risk, with prednisone dose and age as additional factors. Individuals developing diabetes after transplantation had a 7.2-fold increase in fatal infections, with trends toward worse patient and graft survival. Evidence did not favor any specific therapeutic approach.

Islet transplantation

Ryan et al. (33-LB), Edmonton, Canada, presented an update (as of 1 April 2001) on the glycemic control of 15 patients with islet transplantation (two procedures in 11 and three in 4 patients) using a steroid-free immunosuppression regimen. Acute complications of transplantation included bleeding in two, portal vein thrombosis in one, and puncture of the gall bladder in one; none required surgery. All patients initially became insulin-free with measurable C-peptide. Glycemia deteriorated in four, necessitating insulin treatment after being off insulin for a median of 3 months, although in contrast to their prior glycemic lability, they showed stable glucose without hypoglycemia problems and required approximately one-half their pretransplant daily doses. One other patient requires insulin intermittently and is on oral hypoglycemic agents. The mean of the most recent HbA_{1c} for the 11 patients not requiring

insulin was 5.9%. The immunosuppressive therapy caused hypercholesterolemia in 9 of 15, a rise of serum creatinine in 2 of 15, a rise of urine protein excretion in 3 of the other 13, and acne in 5 of 15. Bretzel et al. (345-PP) reported data on 56 consecutive islet transplants from Giessen, Germany, 35 performed simultaneously with and 21 following renal transplant (SIK and IAK transplanted, respectively); all patients underwent only one procedure as opposed to the two or three in the Edmonton report. At mean 1-year follow-up, islet function (C-peptide >0.5 ng/ml) was seen in 80 and 47%, respectively, and the insulin dose decreased from 40 and 45 units pretransplant to 24 and 12 units, with no patient developing severe hypoglycemia, although only 17 vs. 21% were insulin independent. Bretzel mentioned that, excluding their cases, the International Islet Transplant Registry reports 31 and 26% functional islet allograft survival and 7% insulin independence at 1 year after islet transplantation for SIK and IAK recipients.

In an animal study, Lee et al. (286-PP) grafted polyethylene glycol to the capsule of rat pancreatic islets, which were then cocultured with spleen cells, showing decreased immunogenicity and improved survival, with maintained insulin secretory response to glucose, suggesting this as an approach for prevention of rejection. Kojima et al. (307-PP) transfected intestinal stem cells with genes for pancreatic-duodenal homeobox 1, which plays a role in β-cell formation, and for islet factor-1, and exposed the cells to the peptide betacellulin, which promotes pancreatic β-cell differentiation. Implanted in STZ-injected diabetic rats, the cells decreased glucose and increased insulin levels, suggesting another potential approach to islet therapy. Tanwani et al. (1714-P) administered troglitazone or glimepiride to STZ-diabetic rats after islet transplantation. Fasting glucose levels were 78 vs. 139 mg/dl and hyperglycemia developed after 130 vs. 58 days, suggesting enhanced functional survival of the islet graft when insulin resistance was reduced.

Reference

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