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New approaches to type 1 diabetes

ZACHARY T. BLOOMGARDEN, MD

This is the first of eight reports on the American Diabetes Association Annual Meeting and Scientific Sessions, held in San Diego in June. It deals with topics related to type 1 diabetes, including its cell biology and genetic basis, its relation to autoimmune disease and viral infections, and preventive measures directed against the disease. Similarities between type 1 and type 2 diabetes are also covered, and attention is devoted to hypoglycemia and to the effects of C-peptide deficiency in type 1 diabetes.

The Cellular Biology of Type 1 Diabetes

In an effort to evaluate the role of DR3 in the pathogenesis of type 1 diabetes, Kudva et al. (abstract 167; abstract numbers refer to the Abstracts of the 59th Annual Meeting and Scientific Sessions of the ADA, *Diabetes* 48 [Suppl. 1]:1-A550) described a transgenic NOD mouse with the HLA DR3 gene and showed that the antibody response to recombinant human GAD65 is affected by background genes in the context of the HLA gene. Gurr et al. (abstract 170) identified a protein possibly involved in islet-regeneration that was expressed in the islets of a child who died soon after onset of type 1 diabetes. Of 15 patients with recent onset of type 1 diabetes, 11 showed antibodies to similar peptides; only 1 of 14 control subjects showed antibodies to similar peptides. These investigators speculated that overexpression of the islet regeneration protein might act as an autoantigen accelerating the

immune process. Pugliese et al. (abstract 189) reported that the human thymus and peripheral lymphoid organs, such as spleen and lymph nodes, contain peripheral antigen-expressing cells that express islet cell autoantigens, including proinsulin, GAD, and islet cell antibody-512 (IA-2). This may establish tolerance in the thymus during the development of the immune response and help maintain life-long tolerance after the physiological involution of the thymus. Chen et al. (abstract 188) showed increased serum interleukin-12 and interferon- γ in >50% of 32 patients at onset of type 1 diabetes, which suggests that environmental factors, such as viral infection, may initiate onset of diabetes. Inhibition of interleukin-12 secretion or action might benefit autoantibody (AA)-positive individuals at high risk of developing type 1 diabetes.

Dosch et al. (abstract 194) showed diabetes-associated T-cell autoreactivity in 148 patients with recent onset of type 1 diabetes and 268 first-degree relatives, suggesting T-cells as an important factor in the immune response, along with AAs and major histocompatibility complex (MHC) class II (DQ) alleles. The proteasome, or multicatalytic protease complex, plays a role in the immune response by generating antigenic peptides presented by MHC class I molecules. Moreover, the proteasome is involved in many other biological processes, including removal of abnormal proteins, the processing or degradation of transcriptional regulators, and cell differentiation. These

cellular functions are linked to the function of the small polypeptide ubiquitin, which attaches to cytosolic proteins destined for degradation and to certain histones in chromatin and has a role in ribosomal RNA processing and cell surface receptor modification. Type 1 diabetes in both humans and NOD mice has strong genetic linkage to the MHC region compatible with derangement of proteasome processing. Faustman et al. (abstract 168) reported that proteasomes isolated from peripheral lymphocytes of type 1 diabetic patients, NOD diabetic mice, and NOD prediabetic mice showed changes in hydrolytic function, suggesting that proteasome defects may contribute to autoreactivity and possible defects in β -cell susceptibility in type 1 diabetes.

Clinical Data

In an extension of classic data demonstrating the genetic basis of type 1 diabetes, Redondo et al. (abstract 780) analyzed 134 twin pairs from the U.K. and 53 pairs from the U.S., in which one member of each pair had type 1 diabetes. At 25 years of age, 26.9% of the unaffected twins had progressed to diabetes; at 39 years, 41.9% had progressed to diabetes. Pietropaolo et al. (abstract 193) reported on 5,512 first-degree relatives of patients with type 1 diabetes followed for up to 19 years. GAD antibody, two islet cell antibodies (ICAs), and HLA-DQ measurements of 68 type 1 diabetic patients were compared with 371 control subjects. The presence of three antibodies as compared with no, one, and two antibodies conferred a cumulative risk of 76.9% after 12.5 years. However, antibody-negative relatives with four HLA-DQ markers had a 32% cumulative risk, suggesting that additional immunological markers may be needed to improve the ability to predict type 1 diabetes. Steenkiste et al. (abstract 180) reported the risks by age 30 for siblings of type 1 diabetic patients and unrelated control subjects were 6.5 and 0.5%, respectively. For siblings with no, one, and two high-risk haplotypes (either DQA1*0501-DQB1*0201 or DQA1*0301-DQB1*0302), the risks were 2.4, 4.9, and 18.1%, respectively; for control subjects, the risks were

Zachary Bloomgarden is a practicing endocrinologist in New York, New York, and is affiliated with the Division of Endocrinology, Mount Sinai School of Medicine, New York, New York.

Abbreviations: AA, autoantibody; DIPP, Diabetes Prediction and Prevention; DPT-1, Diabetes Prevention Trial for type 1 diabetes; ENDIT, European Nicotinamide Diabetes Intervention Trial; GDR, glucose disposal rate; IAA, insulin autoantibody; IA-2, islet cell antibody-512; ICA, islet cell antibody; MHC, major histocompatibility complex; NE, norepinephrine; OGTT, oral glucose tolerance test; PCA, parietal cell antibody; TRIGR, Trial to Reduce IDDM in the Genetically at Risk; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

0.1, 0.6, and 4.6%, respectively. Having a positive family history of Hashimoto's thyroiditis did not cause significant change in risk. Hoeldtke et al. (abstract 637) studied the question of whether GAD antibodies affect glycemic control and neurological function in recent-onset type 1 diabetes. At 2 and 3 years, those patients with a low titer of GAD65 antibody had HbA_{1c} levels of 8.3 and 8.1%, respectively; those with a high titer had HbA_{1c} levels of 9.7 and 9.2%, respectively. The high-titer group showed more evidence of autonomic neuropathy, independent of glycemia.

Associated Autoimmune Diseases

De Block et al. (abstract 183) reported that 20.9% of 497 patients with type 1 diabetes had parietal cell antibodies (PCAs), which were associated with antithyroid peroxidase antibodies and HLA DR5. Iron deficiency anemia, atrophic gastritis, and pernicious anemia were respectively present in 15.4, 12.5, and 10.5% of those with PCAs vs. 6.9, 2.3, and 0.5% of those without PCAs. Conversely, PCAs were present in 84.6% of patients with pernicious anemia and in 59.1% of patients with atrophic gastritis. Goodman and Edidin (abstract 378) screened 255 asymptomatic children with type 1 diabetes for celiac disease by measuring total IgA, anti-gliadin IgG, anti-gliadin IgA, and anti-endomysial antibody. A total of 13 patients had positive antibodies and had a positive D-xylose test and/or biopsy. In this group and 7 additional patients evaluated because of symptoms, 57% had developed diabetes before 6 years of age. HbA_{1c} and reported hypoglycemia did not decrease after initiation of gluten-free diet. The same authors (abstract 379) screened 227 patients for adrenocortical antibodies. At the initial screening, 10 patients, one of whom later developed Addison's disease, demonstrated elevated antibody titers. Ott et al. (abstract 918) reported that 10.1 vs. 1.4% of thyroid antibody-positive vs. -negative patients with type 1 diabetes had anti-gliadin antibodies, and 16.8 vs. 5.2% had positive adrenal 21-hydroxylase antibodies. Bao et al. (abstract 902) reported that 30.5% of type 1 diabetic patients homozygous for HLA DQ2 had celiac disease-associated transglutaminase IgA AAs. However, Lahtela et al. (abstract 2046) cast doubt on the relevance of screening for celiac disease by reporting that there was no significant change in HbA_{1c} levels, hypoglycemic episodes, insulin doses, or serum lipids with a gluten-free diet in 50 type 1 diabetic patients with celiac disease.

Areas of Overlap Between Type 1 and Type 2 Diabetes

Aizawa et al. (abstract 360) describe nine patients aged 16–36 years with ketosis or ketoacidosis and no ICAs or α -GAD antibodies who became normoglycemic without insulin injection within 3 months. Of the nine patients, two had normal 75-g oral glucose tolerance tests (OGTTs) at 1.3 years. After 3.1–11.7 years of follow-up, three of the remaining nine patients have HbA_{1c} levels <6.5% without medication, and the remaining four require insulin, most in low dosage, which, according to the authors, “exemplifies the complexity of human diabetes and casts strong doubt on the dichotomic etiologic division of common diabetes into type 1 and type 2.” Examining data from the San Luis Valley Diabetes Study, which screened 288 type 2 diabetic patients who were >20 years of age, Hamman et al. (abstract 325) showed that GAD antibodies were expressed in 4.1% of Hispanic patients vs. 2.5% of non-Hispanic white patients. Furthermore, insulin autoantibodies (IAAs) were expressed in 9.9% of Hispanic patients vs. 4.8% of non-Hispanic white patients. Such data are unexpected given the lower incidence of type 1 diabetes among Hispanics. Mohsen et al. (abstract 704) reported that the incidence of type 1 diabetes in young Hispanics versus non-Hispanic whites in El Paso, TX, between 1991 and 1996 was respectively 10.6 vs. 13.2 per 100,000 per year. Takino et al. (abstract 373) discussed 54 GAD antibody-positive patients initially diagnosed as having type 2 diabetes from a group of 2,658. After 2 years, 61% with high-titer and 33% with low-titer antibodies should have received insulin treatment, and Maruyama et al. (abstract 1897) screened 2,040 patients with apparent type 2 diabetes and found 51 GAD65 antibody-positive patients, 5 of whom required insulin. Of the remaining 46 patients, after 1–3 years from the initial screening, fasting and 2-h OGTT glucose and HbA_{1c} levels increased in those kept on sulfonylureas but not in those whose treatment was changed to insulin, which indicates that such patients have a form of type 1 diabetes.

This occurrence may be more common in the U.S. For example, Aviles-Santa et al. (abstract 1911) reported that 12 of 107 patients with secondary oral agent failure in apparent type 2 diabetes had at least one ICA, one IAA, or one GAD antibody, and Pietropaolo et al. (abstract 1903) reported that 12% of 153 patients >65 years of age

had positive GAD65 or IA-2 AAs vs. 1 of 94 nondiabetic control subjects. The patients with islet cell autoimmunity had fibrinogen levels of 343 vs. 318 mg/dl, C-reactive protein 2.96 vs. 2.35 mg/l, and albumin 3.89 vs. 4.08 g/dl, suggesting an inflammatory-autoimmunity component to their disease. Ionescu-Tirgoviste et al. (abstract 1738) reported the annual incidence of type 1 diabetes in Bucharest, Romania, based on either ketoacidosis or need for insulin within 1 month of diagnosis, was 2.7–7.5/100,000 in the population <25 years and increased to 15–21/100,000 at ages 46–65, suggesting that adult onset type 1 diabetes is the predominant form in this area. Hokanson et al. (abstract 187) reported that among 61 Diabetes Control and Complications Trial intensive insulin treatment patients with a family history of type 2 diabetes, BMI increased 3.8 vs. 2.9 in the 521 with a negative family history. Insulin requirements were 0.73 vs. 0.66 U · kg⁻¹ · day⁻¹, HbA_{1c} levels were 7.41 vs. 7.08%, and, at follow-up, triglyceride was 92 vs. 76 mg/dl, cholesterol was 186 vs. 178 mg/dl, and apolipoprotein B was 90 vs. 82 mg/dl, suggesting that these patients had features of the insulin resistance syndrome. Erbey et al. (abstracts 1304 and 1316) studied 20 adult patients at mean age 34 years with type 1 diabetes of 25 years duration using an insulin resistance syndrome score based on triglyceride and HDL cholesterol levels, waist-to-hip ratio (WHR), hypertension, glycemic control, and family history of type 2 diabetes. The glucose disposal rate (GDR) was 10.5, 8.0, and 6.3 mg · kg⁻¹ · min⁻¹ among the three tertiles of insulin resistance score. Visceral fat determined by a computed tomography scan of L4–L5 was the strongest correlate of GDR; WHR, hypertension, and family history of type 2 diabetes were also important factors. Physician-diagnosed angina or confirmed myocardial infarction and overt nephropathy based on albumin excretion rate >200 μ g/min were also predicted by the degree of insulin resistance.

C-Peptide

It is clear that, as a measure of preservation of endogenous insulin, C-peptide distinguishes patients with type 1 diabetes at risk of various complications. Linn et al. (abstract 534) reported the frequency of hypoglycemia in 108 adults with type 1 diabetes followed from onset, 76 of whom received intensive insulin therapy and 32 of whom received conventional insulin therapy. C-peptide-positive vs. -negative patients

on intensive treatment had 0.0071 vs. 0.13 severe hypoglycemic events per year, while conventionally treated patients had similar risk of hypoglycemia with and without C-peptide. The C-peptide-positive and -negative patients had similar hypoglycemic symptoms, but glucagon and epinephrine showed a greater increase with hypoglycemia in the C-peptide-positive patients, while the C-peptide-negative patients showed a greater increase in cortisol levels. Zerbinì et al. (abstract 620) reported that C-peptide levels, corrected for serum creatinine, were approximately twice as high in normoalbuminuric than in micro- or macroalbuminuric patients with type 1 diabetes.

C-peptide may also have direct non-glycemic actions. Sima et al. (abstract 642) treated C-peptide-deficient BB diabetic rats with C-peptide by means of an osmopump. There was no change in blood glucose levels or body weight, but motor nerve conduction velocity increased, and there was evidence of particular improvement in myelinated nerves. Maffi et al. (abstract 234) reported a link between the increase in C-peptide levels after islet transplantation in patients with type 1 diabetes and the degree of improvement in neuropathy based on electromyogram scores, despite a lack of improvement in glycemia. Kunt et al. (abstract 305) incubated whole blood from 20 control subjects and 30 nearly normoglycemic type 1 diabetic patients and showed erythrocyte deformability significantly decreased in diabetic samples and was restored to normal levels by human proinsulin C-peptide at concentrations of 0.6 nmol/l. This effect was abolished by ouabain, which suggests involvement of Na/K-ATPase, a mechanism of C-peptide action inferred from animal studies. Forst et al. (abstract 873) reported that C-peptide infusion in type 1 diabetic patients increased plasma cGMP levels and improved response to acetylcholine as measured by laser Doppler flow, thereby indicating improvement in endothelium-dependent microvascular response.

Prevention of Type 1 Diabetes

At a symposium held at the annual meeting of the American Diabetes Association, Desmond Schatz, Gainseville, FL, spoke on the Diabetes Prevention Trial for type 1 diabetes (DPT-1), one of three major ongoing trials in the illness. The risk of type 1 diabetes is 3% for parents of patients with type 1 diabetes, 5% for siblings, and 8% for offspring of diabetic fathers, but 3% for

offspring of diabetic mothers. Antibodies to GAD, insulin, and islet cells, HLA type, and decreased insulin response all predict diabetes. The risk is ~90% for individuals with antibodies in early childhood, but decreases to ~20% when antibodies are found later in life. In NOD mice, insulin administered either parenterally or orally decreases both the development of diabetes and histological evidence of islet inflammation. Pilot human studies suggest that administering insulin before onset of diabetes prevents the disease. The DPT-1 will study whether insulin treatment of non-diabetic relatives of type 1 diabetic patients can delay or prevent onset by using parenteral insulin in 340 relatives at high (>50%) predicted risk and oral insulin in 490 relatives with 26–50% risk. Thus far, 69,827 people have been screened for antibodies and 65,531 analyzed, with 3.86% positive. Of those screened, 29, 33, and 39% are respectively <10, 10–20, and >20 years old. The study began in 1994; 274 and 219 relatives of type 1 diabetic patients have been randomized to the parenteral and oral insulin groups, respectively. Prior to randomization, 273 of 2,515 ICA⁺ screened individuals had already developed diabetes, confirming the risk of the members of this group. Whether insulin acts as an immunomodulator or a tolerogen or causes β -cell rest is uncertain. ICAs and GAD antibodies are not affected by treatment, and insulin antibodies tend to increase. Schatz quoted Churchill: "It is not even the beginning of the end. But it is perhaps the end of the beginning." As far as safety, there has been mild hypoglycemia in the oral as well as the parenteral insulin treatment groups, the former suggesting dysregulation of insulin secretion.

Palmer et al. (abstract 1,289) studied the effect of the DPT-1 intravenous insulin infusion on endogenous insulin secretion and on postprandial glucose tolerance. In the parenteral insulin arm, those non-diabetic subjects at high risk of future type 1 diabetes who were randomized to active treatment received daily subcutaneous insulin and a 4-day intravenous insulin infusion yearly. Intravenous insulin suppressed fasting and 2-h postprandial C-peptide to 0.19 vs. 1.17 and 3.49 vs. 5.02 ng/ml with corresponding glucose levels of 71 vs. 92 and 214 vs. 160 mg/dl, the latter suggesting temporary postprandial glucose intolerance. Greenbaum et al. (abstract 347) presented evidence of "silent type 1 diabetes"

with fasting glucose <126 mg/dl but 2-h glucose >200 mg/dl in 61 of 571 asymptomatic ICA⁺ subjects screened for the DPT-1 and identified as being at high risk because they had IAAs or low first-phase insulin secretion. An additional 86 subjects had impaired glucose tolerance with fasting glucose 110–126 mg/dl and 2-h glucose 140–199 mg/dl.

Polly Bingley, London, U.K., discussed the European Nicotinamide Diabetes Intervention Trial (ENDIT). Nicotinamide prevents diabetes and inhibits transplant allograft insulinitis in alloxan- or streptozotocin-treated mice, NOD mice, BB rats, and partially pancreatectomized rats. It promotes islet growth and protects against macrophage-mediated cytotoxicity. In humans, modest C-peptide preservation and decreased diabetes in relatives with high risk of diabetes have been found. Moreover, there was decreased progression in ICA⁺ schoolchildren in New Zealand. Nicotinamide may promote cellular defense and repair mechanisms rather than blocking the autoimmune process directly. Cytokines and toxins increase nitric oxide production, leading to DNA strand breaks, restoration of which requires the enzyme poly-ADP ribose polymerase. Pharmacological levels of nicotinamide activate this enzyme. The enzyme also plays a role in apoptosis and in expression of adhesion molecules and histocompatibility antigens. ENDIT has been designed to determine whether nicotinamide at a dose of 1.2 g/m² body surface area can decrease diabetes by 40% in ICA⁺ family members of type 1 diabetic children. Groups from 23 countries are participating. Thus far, 552 patients have been randomized, and two-thirds are <20 years old. Of the patients, 60% are sibs, 9% are children, and 31% are parents of affected individuals. About 50,000 people were screened, with 1.2–3.2% found to be ICA⁺ (a total of 891 persons), of whom 62% were recruited. Of those individuals <20 years of age, 75% have multiple antibodies; of those patients >20 years of age, 45% have multiple antibodies. Those positive only for ICA and those with the protective HLA type DQB1*0602 are at a lower risk. Of 552 patients entered in the study, 95 developed diabetes before entry; of 115 patients who declined to enter the study, 13 developed diabetes, which again shows the validity of the risk assessment approach.

Hans Åkerblom, Helsinki, Finland, discussed the Trial to Reduce IDDM in the

Genetically at Risk (TRIGR), which aims to determine whether the avoidance of cow's milk protein during the first 6 months of life will prevent the development of diabetes to 6 years of age. In a pilot study in Finland, 173 infants had a parent or sibling with type 1 diabetes and were in a high-risk HLA group. Breast-feeding was neither encouraged nor discouraged. The infants were fed either a cow's milk-based formula or a casein hydrolysate, and 9.0 vs. 2.4% developed ICAs, 9.0 vs. 3.6% developed IAAs, 5.6 vs. 1.2% developed GAD antibodies, and 11.2 vs. 3.6% developed any antibodies, respectively. Pronen et al. (abstract 898) reported on a pilot study of TRIGR among children with a first-degree relative with type 1 diabetes and high-risk HLA alleles for type 1 diabetes. At 9 months of age, bovine serum albumin IgG and T-cell responses to insulin were more prevalent in those fed cow's milk formula than in those fed casein hydrolysate or those exclusively breast-fed. T-cell response to human insulin was present in 9 of 14 infants who were fed cow's milk and in 2 of 17 infants who were exclusively breast-fed. Virtanen et al. (abstract 181) showed a 3.24-fold increase in relative risk of type 1 diabetes among individuals who consumed cow's milk during childhood. Correction for HLA-DQB1 genotype increased the relative risk to 4.83-fold, further suggesting that high consumption of cow's milk during childhood may be diabetogenic in siblings of children with type 1 diabetes.

Harrison et al. (abstract 164) reported that intranasal administration of insulin or B25-Asp insulin, which does not bind to the insulin receptor, induced antidiabetogenic CD8 $\gamma\delta$ T-cells that blocked diabetes transfer in NOD mice. In first-degree relatives with at least two serum antibodies to islet antigens, the same group (abstract 894) found changes in antibodies and in T-cell responses to islet antigens. Kupila et al. (abstract 192) presented data from the Diabetes Prediction and Prevention (DIPP) project, in which 1,121 children with HLA alleles associated with increased risk for type 1 diabetes have been observed since birth for the appearance of ICAs. Of these at-risk children, 0.3, 0.9, 2.1, 2.8, 3.6, and 2.3% tested positive for ICA at the ages of 6, 12, 18, 24, 30, and 36 months, respectively. There are plans for a preventive treatment trial of nasal insulin versus placebo. Muona et al. (abstract 927) showed data from the study that first-phase insulin

responses of children with multiple AAs were lower than those of children who were positive for ICA only and that the response was lower in both children with higher ICA titer and in younger ICA⁺ children. Simell et al. (abstract 916) presented a simulation of the direct costs of type 1 diabetes prevention in the DIPP. The average costs per individual for patient-care aspects of the DIPP protocol in the first and in subsequent years were \$972 and \$456, respectively, which is reasonable given the high cost of type 1 diabetes treatment. The effectiveness of the preventive treatment, however, remains to be established.

Marion Rewers, Denver, CO, discussed future prevention trials, beginning with Henry Ford's dictum, "You can't build a reputation on telling what you are going to do." One-third of type 2 diabetes develops before age 7 years, suggesting the need to follow children from birth. Preventive measures can be directed against the auto-immune process, against early β -cell loss, or against clinical diabetes. The Diabetes Autoimmunity Study in the Young includes a family study and a general population cohort of approximately 600 and 1,000 participants screened by HLA-cord blood-typing. By 1 year of age, 30% of DR3/4⁺ related infants and about 10% of nonrelatives develop antibodies, while other relatives and the general population show little autoimmunity. Pilot trials will be initiated at age 0–6 months, with consideration being given to use of oral insulin, peptide fragments of the insulin molecule, GAD, α -3 fatty acids, proteases, and imuran. An alternative approach may be to change the immunologic environment. For example, enterovirus and rotavirus infections may be related to type 1 diabetes, which is particularly seen, albeit infrequently, in the congenital rubella syndrome, suggesting the potential for vaccine development. The inactive insulin analog B25-Asp may be useful in view of the potential adverse effects of hypoglycemia in very young children. Other studies are using the 9-23 fragment of the B-chain, which is effective in a NOD mouse model. Safety requirements, including normal growth, minimal toxicity, no effect on reproductive function, and adequate testing in children, are very important. Use of gluten-free and cow's milk protein-free diets are other potential approaches. Of course, more than 90% of people who develop type 1 diabetes do not have a diabetic relative. HLA DR3/4, DQB1*0302 children have a 1 in 15 chance

of developing type 1 diabetes. Genetic pre-screening is difficult and expensive, and even if benefits are found in trials in relatives these may not be found in treatment of the general population. Another problem will be in explaining the results of genetic screening to parents in the overall population. A huge sample-size will be required in view of the infrequency of type 1 diabetes, even in populations at highest risk, so that more than 9,000 per group will be needed if the frequency is 2% and the intervention has a one-third benefit. Rewers urged the audience to keep in mind that with all of the proposed approaches contributing to our ultimate goal of preventing type 1 diabetes, "Honest differences," as Mahatma Gandhi pointed out, "are often a healthy sign of progress."

Viral Infection and Type 1 Diabetes

Serreze et al. (abstract 169) showed that in the NOD model, coxsackievirus infection did not initiate T-cell responses against GAD but did accelerate the process once underway, suggesting a model of the interaction between viral illnesses and type 1 diabetes. Honeyman et al. (abstract 278) showed increases in rotavirus antibodies occurring concurrently with the appearance of antibodies to insulin in 83%, to GAD in 70%, and to IA-2 in 67% of 41 unrelated at-risk infants with a parent or sib with type 1 diabetes, suggesting that viral infection may trigger islet autoimmunity in genetically susceptible infants. Eibl et al. (abstract 919) showed that serum antibody levels against two T-cell-dependent antigens, diphtheria toxoid and tetanus toxoid, were significantly decreased in 17 patients with type 1 diabetes compared to 16 control subjects. Serum levels of T-cell independent antibodies against the capsular polysaccharide antigens of *Haemophilus influenzae* type b or *Streptococcus pneumoniae* were within normal range. This may have bearing on the pathogenesis of the illness, and may suggest that current vaccination schedules do not adequately immunize patients with type 1 diabetes. Tuomilehto and Karvonen (abstract 698) showed no increase in risk of type 1 diabetes in relation to *Haemophilus influenzae* vaccination in 59,238 children vaccinated in infancy vs. 57,114 vaccinated at 24 months, which suggests earlier reports of an association to be erroneous. In an interesting further vaccine-related study, Baik et al. (abstract 1569) evaluated Bacille de Calmette Guerin (BCG) vaccination in 29

newly diagnosed patients with type 1 diabetes of <4 months duration. Only 2 of 16 vaccinated patients but none of the 13 unvaccinated patients entered clinical remission for 24 months with a trend to increased postprandial C-peptide in the vaccinated group.

Hypoglycemia

Weiss et al. (abstract 178) simulated the changes in life expectancy and costs of intensive versus conventional therapy, addressing whether hypoglycemic events affect outcome in light of the fact that intensive insulin therapy decreases microvascular complications but increases severe hypoglycemic events by threefold. For a cohort of 20-year-old white males with type 1 diabetes of 5 years' duration, the mean expected lifetime costs of severe hypoglycemic episodes amount to \$1,552 with conventional therapy and \$4,820 with intensive therapy. Total lifetime costs of insulin therapy and long-term diabetes complications were \$215,000 for conventional therapy and \$236,000 for intensive therapy. The life expectancy was increased from 46.6 years with conventional therapy to 49.3 years with intensive therapy. The authors pointed out that the survival benefit from intensive therapy would be nullified if the case fatality rate of severe hypoglycemic events exceeded 1 in 240, and stressed the importance of understanding and developing new ways of addressing this complication. Koyama et al. (abstract 216) showed that dogs with carotid body resection had a fall in basal and insulin hypoglycemia-induced glucagon-secretion with decreased glucose production in response to the low blood glucose, suggesting a physiological role of the carotid bodies in the detection of hypoglycemia and subsequent glucagon response. Borg et al. (abstract 239) perfused rats' ventromedial hypothalamii with 2-deoxy-glucose to cause local glucopenia. With propranolol, the rise in plasma glucose was reduced by 65% in association with an 87% lower rise in plasma epinephrine, suggesting a mechanism of the hypothalamic role in response to hypoglycemia. Fanelli et al. (abstract 243) compared the response to hypoglycemia of 6 patients with Addison's disease with and without acute cortisol replacement with that of 10 control subjects. Glucagon and epinephrine responses without cortisol replacement were respectively

13 and 33% of normal, with decreased adrenergic symptoms of heart pounding, tremor, and anxiety, although with similar cholinergic symptoms of sweating, hunger, and paresthesias. Neuroglycopenic symptoms were greater and cognitive function was severely impaired. Acute cortisol replacement normalized glucagon and cognitive function responses. By using metyrapone to produce acute cortisol deficiency in normal subjects, Pampanelli et al. (abstract 304) showed that there was in contrast a greater glucagon and epinephrine response to hypoglycemia, but that cognitive function deterioration was still present, suggesting the importance of glucocorticoids in preserving the function of the central nervous system during hypoglycemia. Heise et al. (abstract 303) administered glyburide at a dose of 3.5 mg during a euglycemic clamp to healthy volunteers receiving enalapril or placebo. The subjects showed a 37% increase in glucose infusion requirement with a 20% increase in insulin secretion, confirming the potentiation of hypoglycemia with ACE inhibition. Hess and Beebe (abstract 1341) compared bedtime snacks containing 30 g carbohydrates alone with snacks containing 30 g carbohydrates and 14 g protein. Fasting glucose fell from 132 to 112 mg/dl with carbohydrate only and increased from 120 to 143 mg/dl with the combined snack, suggesting benefit for patients at risk of nocturnal hypoglycemia. Zinker et al. (abstract 1353) showed that 75 g raw corn starch increased blood glucose 27 vs. 67 mg/dl with oral glucose in 15 nondiabetic healthy subjects. The adjusted area under the glucose curve was reduced 53% from 0–3 h but was increased 27% from 3–4 h with overall 0–4 h levels similar with both substrates.

Gonder-Frederick et al. (abstract 529) found that of 11 patients with a history of recurrent severe hypoglycemia who had experienced an event within 72 h, there was only 3% accuracy in ability to detect mild hypoglycemia induced by insulin infusion vs. 19% accuracy of 14 patients without recent episodes and 21% accuracy in 18 patients with no past episodes. Those with recent hypoglycemia also had lower epinephrine levels. Adrenergic and neuroglycopenic symptoms and ratings of perceived need to self-treat were lower in both hypoglycemia groups than in those without such a history. Burge et al. (abstract 241) studied eight patients with type 2 diabetes and

HbA_{1c} levels of 11.4% treated with glyburide at a dose of 20 mg daily before and after a 1-week period of intensive treatment. Mean capillary glucose was reduced from 177 to 138 mg/dl. The glucose thresholds for epinephrine release and symptoms during insulin-induced hypoglycemia were decreased from 67 to 57 mg/dl and from 68 to 46 mg/dl, potentially increasing the risk of severe hypoglycemia. Davis et al. (abstract 524) reported that two 5-min episodes of hypoglycemia were as effective as two 90-min episodes in blunting epinephrine, glucagon, growth hormone, pancreatic polypeptide, glycerol, free fatty acid, and muscle sympathetic nerve activity responses to subsequent hypoglycemia in nondiabetic individuals. Davis et al. (abstract 1590) also studied patients with type 2 diabetes and showed that a 2-h period of hypoglycemia decreased these responses and their symptoms similarly to a second episode the next day. Tate et al. (abstract 533), from the same group, reported that a 2-h morning episode of hypoglycemia decreased epinephrine, glucagon, pancreatic polypeptide, glucagon, and symptomatic response to an afternoon episode of hypoglycemia in normal subjects but did not affect norepinephrine (NE) or growth hormone responses. Paramore et al. (abstract 523) demonstrated that healthy adults made hypoglycemic twice on the previous day have ~30% lower epinephrine and norepinephrine responses to an episode of hypoglycemia but have similar levels of forearm NE release, suggesting downregulation of adrenal medullary but not of the sympathetic neural effects. In a fascinating study, Jacob et al. (abstract 238) studied diabetic rats that either had chronic hyperglycemia or were exposed to recurrent hypoglycemia. Basal plasma glucose levels were 18.0 and 16.0 in the two groups vs. 8.1 mmol/l in control subjects without diabetes, and levels were 3.1, 3.5, and 3.2 mmol/l during hypoglycemia. Brain extracellular fluid glucose levels measured with microdialysis probes were 3.4, 4.7, and 1.5 mmol/l basally, decreasing to 0.09, 0.06, and 0.22 mmol/l with hypoglycemia. Brain lactate levels were 2.4, 3.8, and 2.6 mmol/l during hypoglycemia in the three groups. Thus, previous recurrent hypoglycemia does not cause resistance to neurological dysfunction during hypoglycemia by increasing brain glucose levels but may involve use of alternate fuels such as lactate.