Type 1 Diabetes and Hypoglycemia

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Type 1 Diabetes and the β-Cell

Matthias von Herrath (La Jolla, CA) received the American Diabetes Association (ADA) 2008 Outstanding Scientific Achievement Award for his research on approaches to β-cell–specific immune interventions for type 1 diabetes. He began with a discussion of the evidence of a role of viral infections in the development of type 1 diabetes. In experimental models, viral infection may either accelerate or retard the immunologic process leading to type 1 diabetes. He pointed out that although type 1 diabetes is clearly an autoimmune disease, because a pancreas transplanted from an unaffected to a type 1 diabetic identical twin is associated with an immune response and rejection, the cause of autoreactivity is uncertain. Environmental factors are likely implicated: viral and also nutritional. Furthermore, the degree of islet inflammation in type 1 diabetes is rather mild, which may shed light on how viral infections might contribute to the disease process because only 3–4% of islets in pre-diabetic patients—and not a great deal more at the time of diagnosis—are affected by insulitis. Approaches to prevention might include modification of genes that predispose to diabetes and their gene products or modification of environmental factors, but emerging evidence suggests that type 1 diabetes is polygenic, with protective as well as enhancing genes, not all of which can suitably be altered.

Curing type 1 diabetes might be accomplished with an unlimited β-cell source, perhaps from stem cells, to make islet transplantation more generally feasible. β-Cell augmentation may be an intermediate goal for which islet transplant protocols not flawed by loss of islets after 3–4 years must be developed, perhaps by eliminating preexisting autoaggressive T-cells. Ongoing trials to prevent and cure recent-onset type 1 diabetes with immune-based and combinatorial therapies need to achieve durable immune suppression while at the same time preserving overall immunity to prevent malignancy and infection ultimately, an approach must be developed to strengthen the body's own β-cell immunoregulatory responses.

Addressing the issue of the contribution of viral infections, von Herrath reviewed the hypothesis that viruses may trigger or enhance type 1 diabetes, noting that viruses can directly infect and lyse β-cells (1). Epidemiological evidence suggests that such rapid cases of type 1 diabetes are uncommon. An alternate hypothesis, that viruses mimic β-cell antigens, appears unlikely, as viral infections do not appear to precipitate type 1 diabetes in otherwise intact animals (2). Rather, he suggested the "fertile field" hypothesis that, in the setting of genetic predisposition to anti-islet autoimmunity, a viral infection can "push a prediabetic animal" into diabetes status. Upregulation of major histocompatibility complex (MHC) class I molecules and interferon-α may be found in human islets in the absence of immune infiltration, which is possibly a signature of viral infection, von Herrath explained. The absence of inflammatory infiltrate suggests that this is directly caused by viral infection, which further supports the notion of the virus' persistence because these markers typically downregulate rapidly after infections resolve. As a consequence of virally induced, interferon-dependent increased MHC class I expression by β-cells, autoaggressive CD8 cytotoxic T-cells are seen in islets in a rodent model (3), along with β-cell destruction, while β-cells not expressing MHC I "are invisible to the immune system." Thus, viral infection may lead to type 1 diabetes by making an underlying autoimmune state manifest. von Herrath concluded that specific β-cell trophic viral infections "do not cause but substantially contribute" to the development of type 1 diabetes. Human islets can in this setting express MHC I and interferons even without an inflammatory infiltrate in individuals genetically at risk.

Von Herrath described, however, another phenomenon, where under certain circumstances viruses can prevent type 1 diabetes, supporting the "hygiene hypothesis" that type 1 diabetes occurs in developed countries where the immune system has a lesser opportunity to be appropriately trained. He reviewed a study in which acceleration versus abrogation of diabetes in an animal model depended on the specific time of infection, with infection before or after a specific time preventing rather than causing diabetes in a type 1 diabetes–prone animal model. Regulatory T-cells reduce the immune response, augmenting production of $CD4/CD25^+$ T-cells leading to production of interleukin (IL)-4 and IL-10. In diabetes-prone models, administration of systemic viral infection–induced regulatory T-cells to at-risk mice will transfer the protective effect, with suppression of CD8 T-cell response to viral infections in part dependent on transforming growth factor (TGF)-β production. Viral infection also increases IL-10 and interferon-γ, which usually play proinflammatory roles. Thus, TGF-β and other protective factors including tumor necrosis factor (TNF)-α downregulate the antiviral immune response, which normally occurs as a virus is being cleared to reduce the systemic immune response. The contraction of the antiviral immune response "pulls along with itself the cells that are attacking the islets." The two components for clinical treatment will be elimination of autoreactive T-cells and the achievement of long-term tolerance through induction of regulatory T cells. Combination therapies will be needed to increase efficacy while reducing adverse effects of immunosuppression. von Herrath reviewed the effect of anti-CD3 antibodies and vaccination with β-cell antigens and found that they have limited effect but appear safe, suggesting that the combination of both approaches may be more effective.

Noting that induction of effector T-cells (Teffs) and regulatory T-cells (Tregs) occurs during infection, inflammation, and autoimmunity, von Herrath addressed the understanding of "switch factors" such as specific cytokines that will favor Tregs rather than Teff "to recruit your own im-

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mune system to do the job." He showed a study of subcutaneous administration of GAD leading to activation and proliferation of Tregs. Cytokines such as TGF-β, IL-4, and IL-10 can modulate this process, generating antigen-presenting cells that reduce Teffs and augment generation of Tregs. One does not need, then, to use the precise autoantigen driving the immune response to produce regulatory T-cells leading to long-term tolerance while avoiding systemic side effects. In animal models, it is possible to show complete and permanent protection with administration of oral insulin, with peri-insulitis representing recruitment of protective Tregs. Not all β-cell proteins are suitable for inducing Tregs, so for human treatment, it may be necessary to develop tools for determining which islet antigen and what dosage are optimal for a given patient, as well as to define the appropriate time for administration. Dose appears to play a crucial role, with administration of porcine and human insulin in animal models appearing protective only in a specific dosage range. Intranasal insulin protects young mice but precipitates diabetes in older mice, suggesting the importance of timing, although it may be possible with combination therapies or use of suitable adjuvants to ensure that regulatory but not islet-attacking T-cells will be produced.

von Herrath again noted the potential synergy between anti-CD3 and antigen-specific immunotherapy, suggesting that such an approach may circumvent side effects and enhance efficacy, with encouraging results in animal studies showing reversal of recent-onset diabetes—which is much more difficult to attain than prevention. Hyperglycemic mice are refractory to combination therapy, suggesting that optimal glycemic regulation characterizes individuals with potential benefit either as a marker of the degree of severity of the diabetes or because of intrinsic benefit of improved glycemia. Both arms of the combined treatment increase Treg and result in loss of aggressive CD8 T-cells. In an animal model, increased induction of CD4/CD25 Tregs and decreased CD8 Teff in spleen and pancreatic lymph nodes were seen after combined treatment. In a small clinical study, type 1 diabetic patients with higher IL-10 and Tregs had better subsequent glycemic control, suggesting that vaccines increasing Tregs might improve glycemia. In a study with intransal proinsulin, proinsulin-specific adaptive Tregs were seen, with enhanced disease prevention when proinsulin immunization was combined with a short course of anti-CD3. Other combinations of immunization with systemic immune modulators have been tested, and the combination of oral and intranasal insulin showed synergy as well.

In silico simulation of optimal treatment regimens may allow optimization of experimental and, later, treatment parameters. In two ongoing intranasal insulin trials, the one with daily administration appears ineffective, while less frequent dosing may be more effective—an outcome predicted by such a simulation. Treatment of type 1 diabetes will require greater availability of human pancreases and better in vivo imaging of pancreatic islets. von Herrath suggested the need to test combination therapies before single drugs have been approved, given his perception that single treatments for type 1 diabetes will be ineffective. The development of biomarkers to predict success of an intervention would be useful, with longitudinal T cell studies potentially such markers. "The ultimate goal," von Herrath concluded, "should be an early childhood vaccine."

In a lecture at the Mount Sinai Diabetes Conference on 23 October 2008, Kevin Herold (New Haven, CT) discussed anti-CD3 treatment of type 1 diabetes. Recalling the classic concept that type 1 diabetes represents the summation of many years of progressive β-cell destruction, with the disease becoming clinically manifest after loss of almost all insulin production, he suggested that now it appears that between 30 and 50% of β-cells remain present at the onset of the disease—far more than thought previously. This offers the possibility that immune treatment may markedly ameliorate the subsequent course, given the close association between endogenous insulin secretory capacity and glycemic control. CD3 is the principal T-cell antigen recognition receptor, and CD4 and CD8 are additional T-cell molecules involved in antigen recognition. Histological examination of islets in human type 1 diabetes involves $CD8^+$ and $CD3^+$ T-cells and relatively few CD4 T-cells (4) in contrast to the findings in the nonobese diabetic (NOD) mouse (a commonly used animal model), implying that treatment approaches based on the model may not readily be extrapolated to man.

The finding of autoantibodies to multiple islet antigens, including GAD, insulin, insulinoma-associated protein 2, and anti-islet cell antibodies is typical at clinical diabetes onset, although these antibodies are not required for β-cell destruction, for which the main effectors are reactive T-cells. The approach that Herold has taken is to use antibodies directed at the ε-portion of CD3, which lead to disease reversal with a peri-islet regulatory lymphocyte infiltrate seen histologicaly. The initially used anti-CD3 preparation, muromonab, is a mouse antibody that causes toxicity from T-cell activation; currently used modified humanized molecules do not show Fc binding leading to far less T-cell proliferation and interferon-γ production, with higher levels of IL-10 and only transient T-cell depletion. In 21 new-onset type 1 diabetic patients treated for 14 days, TNF-α and IL-5, -6, and -10 levels rose, with a mild cytokine-release syndrome of headache, fever, and rash occurring and one patient developing thrombocytopenia. Insulin requirements were stable with treatment while progressively increasing in control patients. C-peptide levels fell by 50% at 1 year and by 75% at 2 years in control subjects but were stable or even increased at 1 year in 15 of the treated patients (5), though then decreasing 50% at 2 years, suggesting that a second course of treatment at 1 year might be appropriate (an approach now being studied).

Another set of studies will involve treatment of pre-diabetic relatives with abnormal glucose tolerance and multiple positive autoantibodies, and treatment of patients with type 1 diabetes present for 4–12 months is also being explored. $CD8^+$ T-cell levels increase with treatment, indicating the presence of regulatory T-cells that appear to reduce the activity of cytotoxic T-cells. TNF-α may be responsible for this effect, which can be blocked in vitro by incubation with anti–TNF-α. Herold showed evidence that as a result of anti-CD3 treatment, rather than elimination of diabetes-specific T-cells reactive to islet autoantigens, the number of such cells rise over the first 3 months after treatment. These may be markers of $CD8^+$ regulatory T-cells. Studies with otelixizumab, a related anti-CD3 compound, have shown similar effects, and anti-CD20 is being studied in animal models.

A final question is whether β-cell regeneration can be demonstrated after anti-CD3 treatment. In NOD mice, it appears that ~10% of β-cells are newly produced from precursor elements, with many of the remaining new cells appearing to be generated from previously dysfunctional β-cells expressing GLUT2 but not insulin. Herold noted that exenatide, rather than increasing β-cell regeneration, seems to increase insulin content of the previously dysfunctional cells, thus appearing to be a

promising approach to treatment of early type 1 diabetes. There may be an effect of modest levels of postprandial hyperglycemia increasing β-cell regeneration; however, above a certain level of glycemia, glucose toxicity effects predominate.

Several studies presented at the ADA meeting shed further light on causes and approaches to treatment of type 1 diabetes. Ferrannini et al. (abstract 150) analyzed characteristics of type 1 diabetes development in 325 islet cell autoantibody-positive, nondiabetic, first-degree relatives from the Diabetes Prevention Trial-1 study, finding that among the 113 who had developed diabetes, impaired β-cell glucose sensitivity rather than a deficiency in absolute insulin secretion rate was the main predictor of progression, with accelerated reduction in this parameter shortly before onset of diabetes. Keenan et al. (abstract 173) found that 31% of 276 individuals with type 1 diabetes for >50 years had either GAD or insulinoma-associated protein 2 antibodies in association with higher C-peptide levels, suggesting residual insulin producing cells. One of the C-peptide–positive patients required treatment with an immunosuppressant (mycophenolate mofetil) for an intercurrent illness and had a 4.7-fold increase in peak C-peptide and a 50% reduction in daily insulin dosage, raising the intriguing possibility of benefit of immune modulation many years after type 1 diabetes onset. Luo et al. (abstract 1596) reported that coculture of human islets with allogenic bone marrow cells stimulated islet growth, reduced IL-1β and β-cell apoptosis, and increased PDX-1 expression, which suggests increased β-cell regeneration. Coad (abstract 1618), noting that β-cells have been observed in the biliary system of normal mice and that there is similar embryological origin of islets and biliary epithelium, cultured mouse adult gall bladder epithelium with adenoviral infection to express the islet transcription factors showing insulin mRNA and protein with increase in release in response to glucose. Brown et al. (abstract 227) showed progressive increase in glucagon secretion during a meal accompanying the reduction in C-peptide secretion during the first year after diagnosis in 23 type 1 diabetic patients (abstract numbers refer to the ADA Scientific Sessions, *Diabetes* 57 [Suppl. 2], 2008).

Understanding of the normal β-cell and of the β-cell in type 2 diabetes will ultimately lead to improvement in the ability to treat all forms of diabetes. Saisho et al. (abstract 1,588) studied pancreas at autopsy within 12 h of death from 105 nondiabetic lean subjects aged 20–100 years and found that despite marked atrophy of the acinar pancreas from age 60 years with a corresponding increase in pancreatic fat, mean β-cell mass remained remarkably constant, implying the existence of regulatory factors preserving β-cells and that the increased incidence of type 2 diabetes with aging is not due to loss of β-cell mass. From the same group, Minh et al. (abstract 4) obtained pancreata at autopsy from six nondiabetic pregnant women and nine nonpregnant women, matched for age and prepregnancy BMI, showing a tripling of β-cell mass from week 20 to 40 of gestation with significantly more small islets and increased numbers of cells positive for insulin in pancreatic ducts without change in β-cell size. Horowitz et al. (abstract 246) showed opposing effects of prolactin and glucocorticoids on β-cell genes including *FoxO1*, *PGC1α*, *PPARα*, and *CPT-1* and on β-cell GLUT2 expression, insulin production, and fatty acid oxidation, suggesting a role in the preservation (and perhaps expansion) of β-cell mass and function during pregnancy. Clark et al. (abstract 1,604) examined lipofuscin body accumulation (a feature of aging that occurs in long-lived, postmitotic cells such as neurons and cardiac myocytes) in islet β-cells obtained at surgery from biopsy specimens of 42 nondiabetic and six diabetic individuals and found a linear increase in lipofuscin area with age in islets from both diabetic and nondiabetic patients, suggesting reduction in turnover and neogenesis of β-cells with age. At age <20 years, 58% of cells did not contain lipofuscin, while at age >40 this was only seen in 15% of cells. The authors commented that islets transplanted from older donors should be studied to ascertain whether insulin secretory function decreases.

Hypoglycemia

Smith et al. (abstract 577) compared 31 and 19 insulin-treated diabetic patients who were aware and unaware of hypoglycemia and found 75 and 43% adherence to recommendations for changes in treatment regimens, respectively, suggesting that purely educational efforts may be insufficient to reduce behavioral and treatment patterns leading to such episodes. Choudhary et al. (abstract 580) studied 87 type 1 diabetic patients over a 9–12 month period and found those with impaired hypoglycemia awareness to have a 3.7-fold greater likelihood of severe hypoglycemia than those without impaired awareness and to have 1.4 vs. 0.6 blood glucose levels <65 mg/dl per week on home capillary glucose monitoring, respectively; 5-day continuous glucose monitoring at the end of the study, however, failed to show significantly more episodes of glucose <55 or <40 mg/dl. Marrett et al. (abstract 586) analyzed self-reported weight gain among 2,008 type 2 diabetic individuals who were not receiving insulin and were participating in the U.S. National Health and Wellness Survey 2007. Weight gain during the prior year of 10–20 pounds was reported by 47% and of >20 pounds by 25% and correlated with experience of more severe hypoglycemia, worry about hypoglycemia, and reduced satisfaction with treatment.

A number of approaches are being developed to address issues related to hypoglycemia. Page et al. (abstract 15) studied 10 type 1 diabetic patients during hypoglycemia either with or without ingestion of medium chain triglycerides and found that the supplement improved verbal memory during hypoglycemia without differences in glucose or counterregulatory hormone levels; plasma ketones and free fatty acids were elevated during hypoglycemia in those receiving the supplement. They (abstract 22) found that glucagon decreases in response to a mixed meal or sulfonylurea in nondiabetic individuals but increases in type 1 diabetic patients; Cooperberg and Cryer interpret this to show that intraislet insulin (perhaps among other β-cell secretory products) normally suppresses α-cell glucagon secretion, whereas in the absence of β-cell secretion the α-cell stimulatory effects of nutrient and the sulfonylurea become manifest. Leu et al. (abstract 20) found that, as expected, an episode of hypoglycemia reduced the counterregulatory response to a second episode the next day; this was prevented by infusion of the opioid receptor blocker naloxone during the initial episode, suggesting that treatments blocking opioid signaling may have benefit in individuals with hypoglycemia unawareness. In contrast, Davis et al. (abstract 21) administered the γ-aminobutyric acid-A receptor agonist alprazolam to 31 normal individuals and showed blunting of epinephrine, norepinephrine, muscle sympathetic nerve activity, pancreatic polypeptide,

glucagon, and growth hormone counterregulatory responses during insulin-induced hypoglycemia the next day, with worsening of the blunting induced by hypoglycemia on the prior day. Henry et al. (abstract 19) measured occipital cortical glucose uptake and showed increased levels in a small group of type 1 diabetic patients with hypoglycemia unawareness, suggesting this to be due to increased transport. Puente et al. (abstract 16) subjected rats to three consecutive days of moderate hypoglycemia or control infusion and found that 1 week after subsequent severe hypoglycemia, neuronal damage was greater in the control group. Bree et al. (abstract 17; from the same group) found, moreover, that severe hyperglycemia caused by streptozotocin 14 days previously increased neuronal damage from severe hypoglycemia.

The importance of in-hospital hypoglycemia is increasingly being addressed. Siram et al. (abstract 18) reported that among 1,641 patients admitted to an intensive care unit, glucose levels of <40 mg/dl and, to a lesser extent, 40–69 mg/dl were associated with increased likelihood of acute renal injury and mortality; insulin administration reduced the likelihood of both renal insufficiency and mortality regardless of hypoglycemia, although subcutaneous appeared less likely than intravenous insulin treatment to be associated with improved outcome. Dhir et al. (abstract 579) described 182 in-hospital hypoglycemia episodes typically occurring after 3 days in the hospital, with risk factors including reduced nutritional intake, major organ dysfunction, resolving infection, increasing insulin dose, and decreasing steroid dose in 60, 31, 22, 12, and 4% of cases, respectively. Natoli et al. (abstract 578) analyzed a hospitalization database of 103,813 diabetic patients from 2000–2006 and found that those experiencing glucose <70 mg/dl had a 58% greater likelihood of discharge to a nursing facility, a 7% greater inpatient mortality, and, on average, three additional hospital days with 39% higher cost. Pittas et al. (abstract 500) reported on a meta-analysis of 15 randomized trials of intensive insulin therapy in 8,472 critically ill, hospitalized, nonpregnant adult patients, with glycemic goal 103–186 vs. 139–260 mg/dl, and showed no difference in mortality but a 4.3-fold greater likelihood of hypoglycemia.

Davidson et al. (abstract 576) reported a meta-analysis of nine trials of more than 1,600 type 2 diabetic individuals receiving biphasic insulin aspart versus biphasic human insulin, showing a 55% reduction in major hypoglycemia and 50% reduction in nocturnal hypoglycemia with the former, although fasting glucose was reduced 37% more with the latter agent. A1C and body weight changed similarly. Similarly, Hutchinson et al. (abstract 582) analyzed 1,005 serious hypoglycemia occurrences among 11,813 type 2 diabetic individuals starting insulin treatment and found a 28% reduction in the risk of these events in users of analog insulin compared with users of human insulin who attained similar mean A1C levels of 8.6–8.9%.

Type 1 diabetes epidemiology

Kahn et al. (abstract 317) analyzed monthly distribution of birth dates of 9,146 diabetic patients in the U.S. who were diagnosed at age <20 years and were participating in the SEARCH for Diabetes in Youth Study. Kahn et al. found a 4% excess diabetes incidence in May and a 5% deficit in November, suggestive of early-life environmental exposures that contribute to childhood diabetes.

Tuomilehto et al. (abstract 23) described another temporal trend in type 1 diabetes. Incidence in Finnish children aged <15 years increased from 32.2 to 64.7 cases per 100,000 people in the general population per year in 1980 and 2005, respectively; the rate of increase appears to have accelerated in the 1990s. Johnson et al. (abstract 24) reported a 47% increase in prevalence of diabetes in the province of Alberta, Canada, during the past decade, with a particularly dramatic 93% increase among those age 1–4 years—suggestive of type 1 rather than type 2 diabetes.

Obesity in type 1 diabetes and its overlap with type 2 diabetes and insulin resistance are gaining interest. Shay et al. (abstract 900) found, as expected, that euglycemic clamp insulin sensitivity was approximately twice as great in 15 type 1 diabetic patients compared with that in 42 type 2 diabetic patients. Insulin sensitivity more strongly correlated with BMI, waist size, percent body fat, and triglyceride level in the type 1 than in the type 2 diabetic group, while it was less strongly associated with diastolic blood pressure. Conway et al. (abstract 29) found that baseline BMI was associated with 18-year mortality in 655 type 1 diabetic patients whose mean age and diabetes duration were 28 and 19 years, respectively, at initial observation. During follow-up, however, underweight patients (BMI <20 kg/m^2) had greater mortality, overweight ($25 \leq$ BMI < 30 kg/m^2) was protective, and obesity (BMI $\geq$30 kg/m^2) was neutral. The authors wondered whether the apparent protection from weight gain during follow-up reflected more insulin use, with 7% of patients initially and 84% at 18 years receiving ≥3 insulin doses/day.

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