

GAD-Alum Treatment Seems Effective in Slowing Residual Beta-Cell Function Loss

Significant C-peptide level preservation was found among treated patients.

BY JOHNNY LUDVIGSSON, MD, PhD; AND ROSAURA CASAS, PhD

Type 1 diabetes is associated with significant morbidity and mortality.^{1,2} Reasonable life quality and expectancy can be acquired with modern insulin therapy, but insulin treatment has no effect on the autoimmune process that is the heart of the disease. Maintaining even a small level of residual insulin secretion with stimulated C-peptide levels >0.2 nmol/L may reduce the risk of these complications that occur over time.³

Parallel to the increasing knowledge of the immune process resulting in beta-cell destruction, the possibility of preserving residual beta-cell function or even restoration of sufficient endogenous insulin secretion seems to become feasible. To date, attempts to preserve residual beta-cell function have shown little benefit or have caused too common and/or serious adverse effects and risks.⁴⁻¹² Multidrug immune modulation before or soon after onset of clinical symptoms may become the treatment of choice for children diagnosed with autoimmune diabetes. Although we believe a treatment strategy using anti-CD3 monoclonal antibodies is promising, therapy-related adverse events have been observed.^{13,14}

Among the broad range of potential interventions, specific immunomodulation with autoantigens has been considered,¹⁵ both in the prevention and treatment of type 1 diabetes. Thus, trials including therapies with insulin¹⁶ and Diapep 277¹⁷⁻¹⁹ have attempted to modify the course of the disease. Among the autoantigens identified as targets for specific T- and B- cell responses in type 1 diabetes, glutamic acid decarboxy-

A selective approach using GAD₆₅ as an immunomodulator is very attractive because type 1 diabetes patients exhibit immune responses against this autoantigen.

lase (GAD₆₅) is one of the major pancreatic antigens targeted by self-reactive T cells.²⁰ T cells specific for GAD₆₅ may be important for the initiation of autoimmune diabetes.²¹ A selective approach using GAD₆₅ as an immunomodulator is very attractive because type 1 diabetes patients exhibit immune responses against this autoantigen.²² In mice, GAD₆₅ prevented autoimmune destruction of pancreatic beta-cells.²³⁻²⁶ Even when data from animal studies have provided strong evidence for the use of autoantigens, results of autoantigen administration to humans with autoimmunity have been ambiguous.

A dose-finding study in patients with latent autoimmune diabetes in adults (LADA) demonstrated that two injections with 20 μ g alum-formulated GAD₆₅ preserved residual insulin secretion with no serious adverse effects.²⁷ Based on those findings, to assess the effect of GAD₆₅ in children with type 1 diabetes, a phase 2 study was conducted. We reported results from the 15-month study period followed by 15 months of further observation in the *New England Journal of Medicine* (2008;359: 1909–1920). A summary appears here.

TABLE 1. BASELINE CHARACTERISTICS OF THE PATIENT, ACCORDING TO STUDY GROUP

Characteristic	GAD-Alum (N = 35)	Placebo (N = 34)
Age (yr)	13.8±2.3	12.8±1.9
Insulin (ultralente, regular, and NPH)	8.8±1.8	8.8±1.9
A1C (%)	15.5±2.8	16.1±3.2
Sex — no. (%)		
Female	25 (71)	18 (53)
Male	10 (29)	16 (47)
HLA-DQA2 genotype — no. (%)		
High risk	25 (71)	18 (47)
Moderate risk	9 (26)	7 (21)
Low risk	1 (3)	9 (26)
Current stage of genital development (Tanner stage) — no. (%)		
1	8 (23)	7 (21)
2-3	8 (23)	10 (29)
4-5	25 (71)	17 (50)
Fasting C-peptide — nmol/liter	0.33±0.18	0.33±0.23
Stimulated C-peptide AUC — nmol/liter/2 hr	1.34±0.57	1.43±0.87
Body mass index (BMI) — kg/m ²	16.1±1.1	16.7±1.8
Insulin dose — U/kg of body weight/24 hr	0.66±0.18	0.66±0.26
Plasma glucose before MM11 — mmol/liter	5.4±0.3	5.5±0.5
Fasting C-peptide:plasma glucose ratio — ×10 ⁻⁴	46±23	45±20
Anti-GAD autoantibody titer — IU/ml	803	803

Plasma glucose values are mean ±SD; AUC, denotes area under the curve; GAD, the 65-kD isoform of glutamic acid decarboxylase; LADA, latent autoimmune diabetes in adults; MM11, a standard vaccine formulation with alum, and MM11 mixed meal tolerance test. To convert values for C-peptide to nanograms per milliliter, divide by 0.53. To convert values for glucose to milligrams per deciliter, divide by 0.0555. The body mass index (BMI) is the weight in kilograms divided by the square of the height in meters. The Tanner stage of genital development can range from 1 to 5, with an increasing score indicating more developed genitalia.

Ludvigsson, et al. N Engl J Med. 2009;359:1909–1920. Table reproduced with permission copyright 2008 Massachusetts Medical Society. All rights reserved.

CLINICAL TRIAL DESIGN

At eight Swedish pediatric clinics, patients aged 10 to 18 years who presented with type 1 diabetes within the previous 18 months were screened for GAD autoantibodies and fasting C-peptide levels >0.1 nmol/L (0.3 ng/mL). Seventy patients were eligible and randomized in double-blind fashion to treatment with 20 μg of GAD-alum (Diamyd; Diamyd Medical, Stockholm) or placebo. All but one patient received two doses of either GAD-alum or placebo, and 69 patients (35 in the GAD-alum group and 34 in the placebo group) were included in the intention-to-treat analysis. Data for the two study groups were similar at baseline, and the distribution of human leukocyte antigen (HLA) genotypes did not differ between the GAD-alum group and the placebo group (Table 1).

Patients also received multiple daily insulin injections with a target A1C level of <6.5%. We sought to evaluate safety and efficacy of GAD-alum treatment versus placebo in preserving residual insulin secretion. The pre-specified primary efficacy endpoint was the change in fasting C-peptide levels from baseline to

month 15. Prespecified secondary efficacy endpoints were changes in fasting and stimulated C-peptide levels and A1C values from baseline to various prespecified time points, up to month 30. Other endpoints included insulin requirement, fasting plasma glucose level, fasting C-peptide:plasma glucose ratio and GAD autoantibody titer.

The primary injection of GAD-alum or placebo was given on day 1 and a boost was administered at 1 month. A 2-hour mixed-meal tolerance test was performed on day 1 and at months 3, 9, 15, 21, and 30. After 15 months, data were unblinded to external statistician but the trial continued double blind for the laboratory, clinicians, patients and parents for a 15-month extension period. T-cell studies were performed under a separate investigator-initiated protocol.

Results from the study with LADA patients²⁷ suggested that 35 patients in each study group would provide a statistical power of 80% to 90% for assessing C-peptide level differences. A prespecified analysis of covariance model was used, in which the change from baseline was taken as the response variable, the study treatment as the explanatory variable, and the baseline value as a covariate. Age, sex, duration of diabetes at baseline, GAD autoantibody titer, and HLA type were identified as factors for additional exploratory analyses and were used to prespecify subgroups.

The null hypothesis was that there was no difference between active treatment and placebo, and two-sided tests were used for all hypotheses. *P* values were not adjusted for multiplicity²⁸ and the hypothesis of no difference between the study groups was assessed at months 15 and 30 of follow-up.

SAFETY

A major concern with antigen-based therapies is the possible outcome of adverse events. The most commonly reported adverse events during the main study period were upper respiratory tract infection, naso-

Ludvigsson, et al. N Engl J Med. 2009;359:1909–1920. Figures reproduced with permission copyright 2008 Massachusetts Medical Society. All rights reserved.

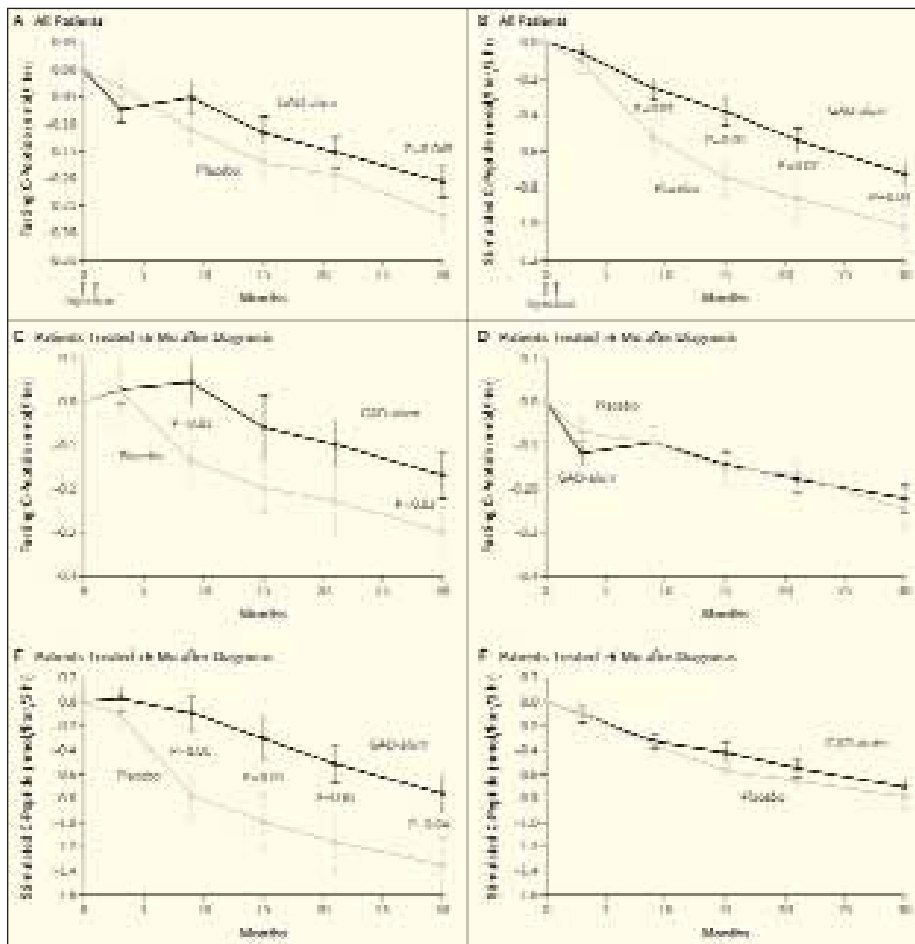


Figure 1. Mean changes from baseline levels of fasting and stimulated C-peptide, according to treatment group and time of treatment relative to diagnosis.

pharyngitis, gastroenteritis, and headache. In two patients in the GAD-alum group the adverse events were determined to be possibly related to study treatment—one patient developed mild hypoglycemia and one developed moderate hypoglycemia. Thus, our results indicate that the treatment was safe and well tolerated by the patients.

Seven serious adverse events occurred in five patients in the GAD-alum group and five occurred in four patients assigned to placebo. In the treatment group, the events were knee trauma, ketoacidosis, lower-limb fracture, and diarrhea, and one patient had ketoacidosis, high A1C, and streptococcal tonsillitis. In the placebo group, events included mononucleosis, ankle fracture, cessation of insulin use, and two episodes of hypoglycemia with seizure in one patient.

PRESPECIFIED EFFICACY ENDPOINTS

Both groups demonstrated a progressive decrease

from baseline in fasting and stimulated C-peptide secretion, indicating a gradual loss of beta-cell function (Figure 1A and 1B). No significant effect of treatment was seen on the change in fasting C-peptide level between baseline and month 15, however, there was a significant effect of treatment on the change in fasting C-peptide level by month 30 ($P=.045$) (Figure 1A) and on the change in the C-peptide: plasma glucose ratio ($P=.02$). Area-under-the-curve (AUC) stimulated C-peptide secretion decreased significantly less in the GAD-alum group versus placebo-assigned patients, by month 15 ($P=.01$) and by month 30 ($P=.04$) (Figure 1B).

Insulin requirement, A1C and plasma glucose levels increased in both study groups during the study, but did not differ significantly between the two groups.

EXPLORATORY ANALYSES OF EFFICACY

Even after adjusting for duration of diabetes, age, gender, and baseline GAD autoantibody levels, the significant effect of treatment on the change between baseline and month 30 in fasting and stimulated C-peptide levels remained. The prespecified subgroups were also evaluated for interaction effects, with only duration of diabetes had a significant influence on study treatment efficacy ($P=.05$ for fasting at month 30 and $P=.03$ for stimulated C-peptide level [AUC], at months 15 and 30).

An exploratory formal analysis of the prespecified subgroups regarding diabetes duration showed that among patients treated <6 months after diagnosis, both fasting and stimulated C-peptide secretion decreased significantly less in the GAD-alum group than in the placebo group by month 30 ($P=.03$ and $P=.04$, respectively) (Figures 1C and 1E). No significant difference was observed between the two groups for patients treated ≥6 months after diagnosis (Figures 1D and 1F).

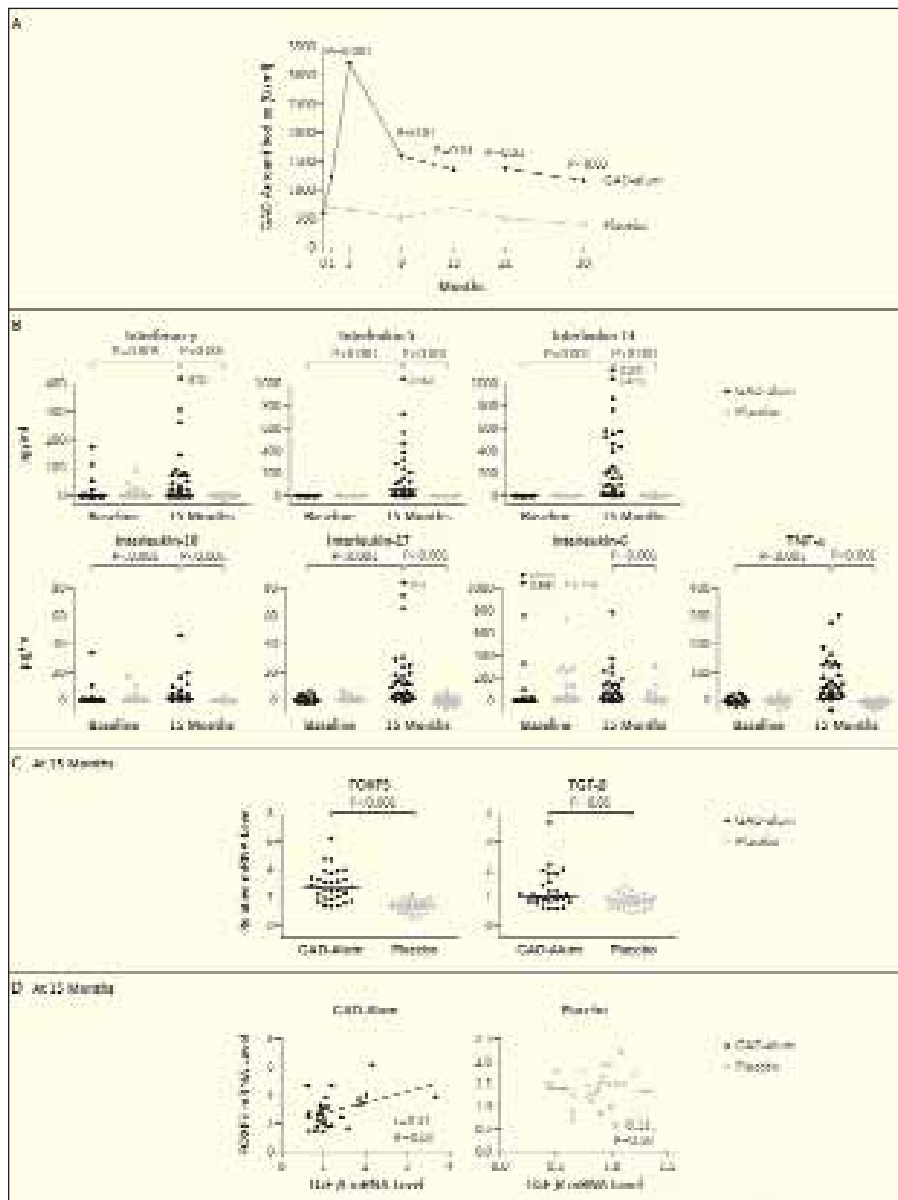


Figure 2. Effects on the immune system.

INDUCTION OF ANTIGEN-SPECIFIC IMMUNE RESPONSES

One of the main challenges in immunomodulating therapies is to obtain long-lived therapeutic outcomes with shorter therapeutic interventions. Thus, the efficiency of the autoantigen to induce a specific T-cell response strong enough should be determinant in the effect of the treatment. In our study it was evident that GAD₆₅ induced an antigen specific T-cell population detectable in peripheral blood. The modulatory effect of the therapy in T-cell immune responses resulted in the induction of a GAD₆₅-specific cell pop-

ulation able to secrete Th1, Th2 and immunoregulatory cytokines (Figure 2 B), which express FOXP3 and TGF-β (Figure 2C). This cellular recall immune phenotype persisted 15 months after the first injection. In treated patients, GAD autoantibody levels rapidly increased, reaching a maximum at 3 months. Levels then decreased, but remained significantly higher versus placebo (Figure 2A). Thus, our results provided evidence for the effect of GAD₆₅ in the induction of specific humoral immune responses to the antigen. More importantly, the treatment was able to induce long-lasting immune responses, still detectable 30 months after the first injection.

It seems reasonable to assume that modulation of the general memory immune responses to GAD₆₅ in the treated children could be the result of activation of a protective specific immune responses towards the autoantigen. A premise for the inclusion of patients in our trial was detectable levels of GADA autoantibodies, indicating ongoing immune responses

toward GAD₆₅. Even though all the patients were able to exert similar GAD₆₅-induced cytokine secretion before the treatment, all the studied cytokines significantly increased in patients receiving GAD₆₅-alum after 15 months.

It is unclear whether immunizing in humans with a preexisting pathogenic response would boost that pathogenic response. Our observations suggest that this is not the case, but rather the responses to GAD₆₅ in the treated diabetics seems to be the result of activation of protective specific immune responses towards the autoantigen.

DISCUSSION

Stimulated C-peptide levels have been considered an endpoint for assessing beta-cell function preservation early in type 1 diabetes. These levels correlate with improved glycemic control and fewer microvascular complications.^{13,14,29} We found no significant effect of GAD-alum treatment on the change in the primary endpoint of fasting C-peptide, but an effect was seen on stimulated C-peptide level. Both fasting and stimulated C-peptide levels declined to a significantly smaller degree in the GAD-alum group than in the placebo group after 30 months. The observed protective effect of treatment on C-peptide secretion was seen only in patients treated <6 months after diagnosis.

In our study, the duration and magnitude of the GAD-alum treatment effect appears similar to that reported for anti-CD3 treatment^{13,14} but without the related adverse events. Especially over the long term, residual insulin secretion affects key clinical outcomes.³ Possible explanations include improved overall metabolic control, reduced fluctuation in blood glucose levels and perhaps increased exposure to C-peptide.³⁰ Our results indicate that two injections of 20 µg GAD-alum may help preserve residual insulin secretion in patients with recent-onset type 1 diabetes. How GAD-alum treatment may work to alter disease progression in type 1 diabetes is unclear. Therapy was otherwise similar in the two study groups, therefore differences in preserved beta-cell function do not appear to be related to more intense insulin treatment or better metabolic control in the GAD-alum group. Although fasting C-peptide level may be influenced by blood glucose level, we found significant C-peptide level preservation before and after adjustment for blood glucose level.

CONCLUSION

Treatment with GAD-alum had an effect on slowing the loss of residual beta-cell function up to 30 months after intervention and was associated with GAD-specific immune modulation. This did not, however, change the insulin requirement. Our study provides preliminary proof of concept; large-scale confirmatory studies with GAD-alum are under way in the United States and Europe. ■

Johnny Ludvigsson, MD, PhD; and Rosaura Casas, PhD, are from the Division of Pediatrics, Department of Clinical and Experimental Medicine, Faculty of Health Sciences, Linköping University, Linköping, Sweden. Dr. Ludvigsson may be reached at johnny.ludvigsson@lio.se. The study was supported by grants from Diamyd Medical and the Swedish Child Diabetes Foundation (Barndiabetesfonden). He disclosed that he has received grant support from Diamyd

Medical for the mechanistic studies and from NovoNordisk for studies on insulin analogues

1. Group. TDCaCTR. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med.* 1993;329:977–86.
2. Bojestig M, Arnqvist HJ, Hermansson G, et al. Declining incidence of nephropathy in insulin-dependent diabetes mellitus. *N Engl J Med.* 1994;330:15–18. [Erratum, *N Engl J Med.* 1994;330:584.]
3. Steffes M, Sibley S, Jackson M, Thomas W. Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care.* 2003;26:832–836.
4. Dupré J, Stiller C, Gent M, et al. Clinical trials of cyclosporin in IDDM. *Diabetes Care.* 1988;11:37–44.
5. Eisenbarth G, Srikanta S, Jackson R, et al. Anti-thymocyte globulin and prednisone immunotherapy of recent onset type 1 diabetes mellitus. *Diabetes Res.* 1985; 2:271–276.
6. Ludvigsson J, Heding L, Liedén G, et al. Plasmapheresis in the initial treatment of insulin-dependent diabetes mellitus in children. *Br Med J (Clin Res Ed).* 1983;286:176–178.
7. Chase H, Butler-Simon N, Garg S, et al. A trial of nicotinamide in newly diagnosed patients with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia.* 1990;33:444–446.
8. Pozzilli P, Visalli N, Signore A, et al. Double blind trial of nicotinamide in recent-onset IDDM (the IMDIAB III study). *Diabetologia.* 1995; 38:848–852.
9. Coutant R, Landais P, Rosilio M, et al. Low dose linomide in type 1 juvenile diabetes of recent onset: a randomised placebo-controlled double blind trial. *Diabetologia.* 1998; 41:1040–1046.
10. Ludvigsson J, Samuelsson U, Johansson C, Stenhammar L. Treatment with antioxidants at onset of type 1 diabetes in children: a randomized, double-blind placebo-controlled study. *Diabetes Metab Res Rev.* 2001;17:131–136.
11. Ludvigsson J, Samuelsson U, Ernerudh J, et al. Photopheresis at onset of type 1 diabetes: a randomised, double blind, placebo controlled trial. *Arch Dis Child.* 2001; 85:149–154.
12. Raz I, Elias D, Avron A, et al. Beta-cell function in new-onset type 1 diabetes and immunomodulation with a heat-shock protein peptide (DiaPep277): a randomised, double-blind, phase II trial. *Lancet.* 2001;358:1749–1753.
13. Herold K, Gitelman S, Masharani U, et al. A single course of anti-CD3 monoclonal antibody hOKT3gamma1(Ala-Ala) results in improvement in C-peptide responses and clinical parameters for at least 2 years after onset of type 1 diabetes. *Diabetes.* 2005;54:1763–1769.
14. Keymeulen B, Vandemeulebroucke E, Ziegler A, et al. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med.* 2005;352:2598–608.
15. Ludvigsson J. Adequate doses of autoantigen administered using the appropriate route may create tolerance and stop autoimmunity. *Diabetologia.* 2009;52:75–76.
16. Skyler J, Krischer J, Wolfsdorf J, et al. Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial—Type 1. *Diabetes Care.* 2005; 28:1068–1076.
17. Huurman V, Decochez K, Mathieu C, et al. Therapy with the hsp60 peptide DiaPep277 (trade mark) in c-peptide positive type 1 diabetes patients. *Diabetes Metab Res Rev.* 2007;23:269–275.
18. Huurman V, van der Meide P, Duinkerken G, et al. Immunological efficacy of heta shock protein 60 peptide Diapep277TN therapy in clinical type 1 diabetes. *Clin Exp Immunol.* 2008;152:488–497.
19. Raz I, Avron A, Tamir M, et al. Treatment of new-onset type 1 diabetes with peptide DiaPep277 is safe and associated with preserved beta-cell function: extension of a randomized, double-blind, phase II trial. *Diabetes Metab Res Rev.* 2007;23:292–298.
20. Baekkeskov S, Aanstoot H-J, Christgau S, et al. Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature.* 1990;347:151–156.
21. Kaufman D, Clare-Salzler M, Tian J, et al. Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature.* 1993;366:15–17.
22. Ellis T, Atkinson M. The clinical significance of an autoimmune response against glutamic acid decarboxylase. *Nat Med.* 1996;2:148–153.
23. Tisch R, Liblau RS, Yang XD, et al. Induction of GAD65-specific regulatory T-cells inhibits ongoing autoimmune diabetes in nonobese diabetic mice. *Diabetes.* 1998; 47:894–899.
24. Jun HS, Chung YH, Han J, et al. Prevention of autoimmune diabetes by immunogene therapy using recombinant vaccinia virus expressing glutamic acid decarboxylase. *Diabetologia.* 2002;45:668–676.
25. Tisch R, Wang B, Atkinson M, et al. A glutamic acid decarboxylase 65-specific Th2 cell clone immunoregulates autoimmune diabetes in nonobese diabetic mice. *J Immunol.* 2001;166:6925–6936.
26. Chen C, Lee W, Yun P, et al. Induction of autoantigen-specific Th2 and Tr1 regulatory T cells and modulation of autoimmune diabetes. *J Immunol.* 2003;171:733–744.
27. Agardh CD, Cilio CM, Lethagen A, et al. Clinical evidence for the safety of GAD65 immunomodulation in adult-onset autoimmune diabetes. *J Diabetes Complications.* 2005; 19:238–246.
28. Pergener T. What's wrong with Bonferroni adjustments. *BMJ.* 1998;316.
29. Steele C, Hagopian W, Gitelman S, et al. Insulin secretion in type 1 diabetes. *Diabetes.* 2004;53:426–433.
30. Wahren J, Ekberg K, Johansson J, et al. Role of C-peptide in human physiology. *Am J Physiol Endocrinol Metab.* 2000; 275:E759-E68.