Antibodies to GAD and Glycemic Control in Recent-Onset IDDM

ROBERT D. HOELDTKE, MD, PHD
KIMBERLY D. BRYNER, MS
GABRIELLA G. HORVATH, MD
MICHELE R. BYERLY, RN
GERALD R. HOBBS, PHD
SANTICA M. MARCOVINA, PHD
AKE LERNMARK, PHD

OBJECTIVE — To analyze the effect of antibodies to glutamic acid decarboxylase (GAD65Ab) and islet cells (ICA512Ab) on glycemic control early in IDDM.

RESEARCH DESIGN AND METHODS — GAD65Ab and ICA512Ab were measured twice in 35 patients (10 male, 25 female; age 10–40 years) initially within 2 years of diagnosis and again 1 year later. The glycosylated hemoglobin was measured one to four times each year, and the average glycosylated hemoglobin for the preceding year was calculated each time the antibodies were measured.

RESULTS — The mean HbA_1 at the time of the initial evaluation was 8.04 ± 0.30 (reference range 4.7–7.3% for nondiabetic patients), the average GAD65Ab index was 0.735 ± 0.306, and the mean ICA512Ab index was 1.94 ± 0.65. The GAD65Ab index correlated with HbA_1 (r = 0.41, $P < 0.025$), whereas the ICA512Ab index did not (r = 0.13). One year later, the mean GAD65Ab index was 0.94 ± 0.34, the mean ICA512Ab index was 1.04 ± 0.40, and the mean HbA_1 was 9.03 ± 0.30. The GAD65Ab index correlated with HbA_1 (r = 0.61 $P < 0.001$), whereas the ICA512Ab index did not (r = −0.06). Stratification of patients into tertiles according to the average GAD65 index revealed, at the follow-up evaluation, that the better glycemic control in the lowest GAD65Ab tertile was accomplished with significantly less insulin (0.43 ± 0.08 U/kg for the lowest tertile vs. 0.71 ± 0.09 and 0.64 ± 0.09 for the middle and highest tertiles, respectively; $P < 0.05$).

CONCLUSIONS — In summary, patients with IDDM and low GAD65Ab have better glycemic control even though they require less insulin. The ICA512Ab index, however, fails to correlate with glycemia.

Autoantibodies to the islet cells (ICA512Ab) and a smaller isoform of glutamic acid decarboxylase (GAD65Ab) are currently recognized as immunological markers of IDDM (1,2), but their relative importance has been disputed (2–4). There is little or no information on the effect of these antibodies on glycemic control. In the present study, we determined GAD65Ab and ICA512Ab levels using standardized radiobinding ligand assays, and provided evidence that, in patients with recently diagnosed IDDM, the presence of GAD65Ab, but not ICA512Ab, signifies a more aggressive disease as indicated by higher glycosylated hemoglobin concentrations and higher insulin requirements.

RESEARCH DESIGN AND METHODS

GAD65Ab

GAD65Ab levels were measured in 35 IDDM patients (10 male, 25 female; age 10–40 years, median age 18 years). The patients were enrolled in a longitudinal study in which GAD65Ab measurements were made to assess their putative relationship to neurological dysfunction (5). The patients have completed two annual evaluations, beginning within the first 2 years of their illness (Table 1). All patients were trained to monitor their glucose at home and adjust their insulin dosage and food intake accordingly. HbA_1 was measured 1–4 times each year (6). The reference range for the nondiabetic population was 4.7–7.3%.

GAD65Ab levels were measured in our radiobinding immunoassay (7,8); they were expressed as a relative index (GAD65 index) using one positive serum (Juvenile Diabetes Foundation World Standard for ICA) and three negative standard sera from healthy subjects, as previously described (9,10). The interassay coefficient of variation was 14.7% (10,11).

The patients were stratified into tertiles on the basis of their GAD65Ab index; differences in glycosylated hemoglobin and insulin dosages and the effect of time were assessed using repeated measures analysis of variance (12).

ICA512Ab

Antibodies to the islet cell antigen, ICA512, were measured under identical conditions as those described for GAD65Ab, using a plasmid containing the ICA512 cDNA (13).

RESULTS

GAD65Ab

GAD65Ab were detected in 30 of 35 patients (86%). The GAD65Ab index at the initial evaluation generally correlated well with that observed 1 year later (Fig. 1) (7). Analysis of the pooled data from the initial and follow-up evaluations revealed a correlation between the average GAD65Ab index and average glycosylated hemoglobin concentrations (r = 0.57, $P < 0.01$) (Fig. 2). The correlation held when data for male subjects (r = 0.67, $P < 0.05$) and female subjects (r = 0.55, $P < 0.005$) were analyzed separately. The correlation between GAD65Ab and HbA_1 was also evident when data gathered at the initial and follow-up evaluation were analyzed separately (Table 2).

Stratification of patients according to GAD65Ab tertiles confirmed that those with

From the Department of Medicine (R.D.H., K.D.B., G.G.H., M.R.B.) and Departments of Community Medicine and Computer Science and Biostatistics (G.R.H.), West Virginia University, Morgantown, West Virginia; and the Department of Medicine (S.M.M., A.L.), University of Washington, Seattle, Washington.

Address correspondence and reprint requests to Robert D. Hoeldtke, MD, PhD, Department of Medicine, P.O. Box 9159, West Virginia University Medical School, Morgantown, WV 26506-9159. E-mail: bmusick@wvudeptmed1.hsc.wvu.edu.

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Abbreviations: GAD65Ab, antibodies to glutamic acid decarboxylase; ICA512Ab, islet cell antibodies.

Table 1—*Clinical characteristics of patients*

	Male	Female
n	10	25
Age at diagnosis (years)	19 (10–37)	19 (10–40)
Disease duration at initial evaluation (months)	10.3 (3–22)	10.5 (2–24)
GAD65Ab index*	1.62 (0.13–11.8)	0.53 (0–2.57)
ICA512Ab index†	2.73 (0–8.99)	0.845 (0–9.0)
HbA_1 (%)‡	8.96 (7.1–12.5)	8.43 (6.0–10.7)

Data are means (range). *Calculated from the GAD65Ab index at initial evaluation and 1 year later; †calculated from the ICA512Ab index at initial evaluation and 1 year later; ‡calculated by taking the mean of the average HbA_1 before the initial evaluation and the average HbA_1 during the year between the initial and final evaluations.

higher GAD65Ab index had poorer glycemic control, both at the time of the initial evaluation and 1 year later (Table 3). Furthermore, patients in the lowest GAD65Ab tertile required lower amounts of insulin than did patients in the other tertiles (Table 3).

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ICA512Ab were detected in 7 of 10 male patients and 13 of 25 female patients. Neither glycosylated hemoglobin (Table 2) nor insulin dosage nor GAD65Ab correlated with ICA512Ab. Patients who were above the median for both ICA512Ab and GAD65Ab had no worse glycemic control than those who were above the median for GAD65Ab only.

CONCLUSIONS — Antibodies to GAD65 are increasingly recognized as sensitive, specific, predictive immunological markers of IDDM (1,7,14). Despite this, their role in the pathophysiology of β-cell destruction is poorly understood. Previous studies of the effect of GAD65Ab on β-cell function have led to conflicting results. Petersen et al. (2) studied a large cohort of IDDM patients and observed that, 1 year after diagnosis, the glucagon-stimulated C-peptide secretion was decreased 30% in $GAD65Ab^+$ patients (2). Similarly, patients with the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome and high GAD65Ab were found to have a lower fasting C-peptide level and less of an insulin response to an intravenous glucose challenge than did $GAD65Ab^-$ patients with this syndrome (15). Studies of C-peptide in other cohorts,

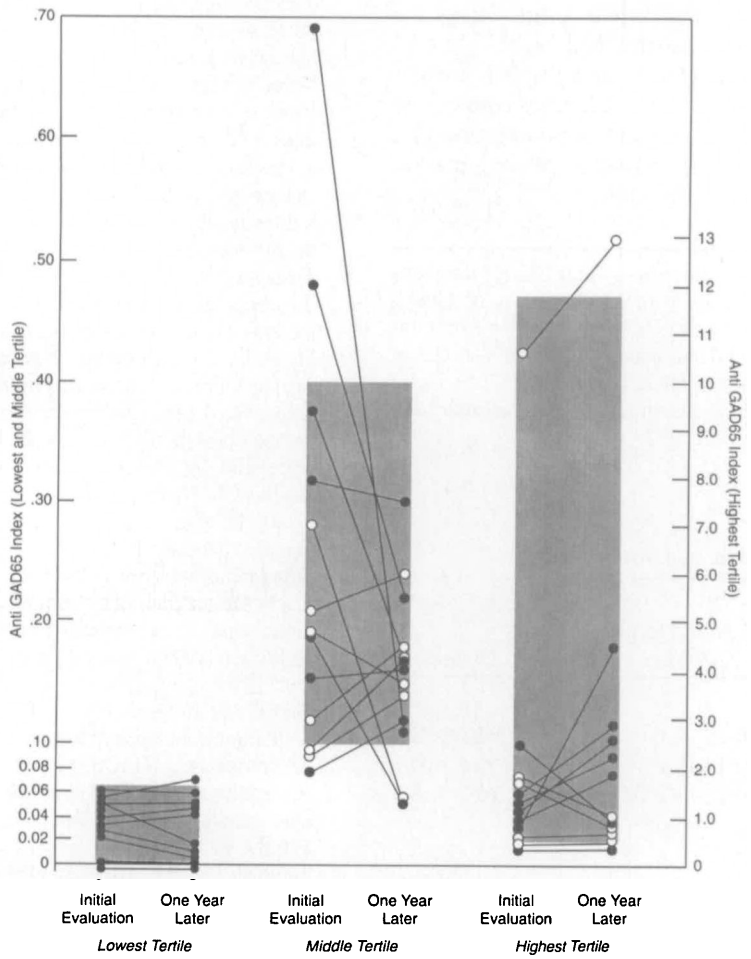

Figure 1—*Stratification of patients according to the average GAD65Ab index. The average GAD65Ab index was calculated for each patient; tertiles were then constructed as illustrated by the shaded areas. Each circle represents an individual patient, and the lines connecting the circles illustrate the change between the initial and subsequent measurements. ○, male patients; ●, female patients. Units for highest tertile (on right) are different from units for other tertiles (on left).*

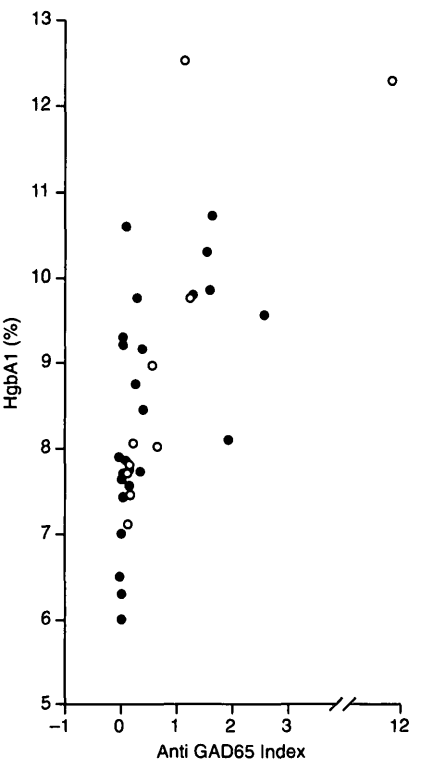

Figure 2—*GAD65Ab index versus glycosylated hemoglobin. The average GAD65Ab index is plotted against the mean of the average HbA_1 from the 1st and 2nd years. Between GAD65Ab and HbA_1, r = 0.57, P < 0.01. Elimination of the patient with marked elevation in GAD65Ab resulted in r = 0.58, P < 0.01. ○, male patients; ●, female patients.*

Table 2—*Correlation of GAD65Ab and ICA512 with glycosylated hemoglobin*

	At initial evaluation	One year later	Average index versus average HbA_1
GAD65Ab versus HbA_1			
r	0.41	0.61	0.57
P	<0.025	<0.001	<0.001
ICA512 versus HbA_1			
r	0.15	−0.06	0.03

however, have failed to establish a correlation between GAD65Ab and insulin secretion early in IDDM (16); one recent small study even suggested that GAD65Ab are associated with β-cell protection (17). It is worth noting that, in studies that failed to confirm a correlation between GAD65Ab and C-peptide (16,17), the patients were very young (mean age <10 years), whereas in our study and that of Petersen et al. (2), the mean age of the patients was 19 and 22 years, respectively. Cross-sectional studies of large cohorts of IDDM patients have indicated islet cell antibodies may mediate aggressive autoimmune processes in young children, whereas GAD65Ab trigger β-cell–directed autoimmunity in adolescents and young adults (18). Thus these antibodies may carry a different significance in different age groups, which may explain the discrepancies in the published studies on the relationship between GAD65Ab and β-cell function (2,16,17).

Unfortunately, C-peptide measurements were not made in our study, which was designed for other reasons. Nevertheless, because insulin requirements are known to vary inversely with C-peptide during the first few years of IDDM (4), it seems likely that the patients with high levels of GAD65Ab have less endogenous insulin secretion than patients with low levels or absent GAD65Ab. Imagawa et al. (19) recently performed pancreatic biopsies on patients recently diagnosed with type 1 diabetes. Those with GAD65Ab had more intense insulitis than those lacking GAD65Ab (19). Two years later, the $GAD65Ab^+$ patients had higher glycosylated hemoglobin and higher insulin requirements (19). These data corroborate our own and support our contention that GAD65Ab play a role in (or are a marker for) β-cell destruction.

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Table 3—*GAD65 antibody index, glycosylated hemoglobin, and insulin dosage*

	GAD65Ab tertiles		
	Lowest	Middle	Highest
GAD65Ab			
Initial evaluation	0.031 ± 0.006	0.251 ± 0.049	1.85 ± 0.81
One year later	0.022 ± 0.009	0.158 ± 0.19	2.55 ± 1.01
Average GAD65Ab*	0.026 ± 0.005	0.204 ± 0.027	2.20 ± 0.64
HbA_1 (%)			
Initial evaluation	6.98 ± 0.36	7.72 ± 0.48	9.21 ± 0.41†
One year later	7.82 ± 0.45	8.70 ± 0.22	10.4 ± 0.58‡
Average HbA_1*	7.40 ± 0.35	8.21 ± 0.27	9.80 ± 0.42‡
Insulin dose (U/kg)			
Initial evaluation	0.352 ± 0.063‖	0.498 ± 0.070	0.619 ± 0.124
One year later	0.434 ± 0.079§	0.711 ± 0.087	0.640 ± 0.094
Average insulin dose	0.393 ± 0.066§	0.604 ± 0.073	0.630 ± 0.105

*Defined in footnote to Table 1; †different from other tertiles, $P < 0.005$; ‡different from other tertiles, $P < 0.001$; §different from other tertiles, $P < 0.05$; ‖versus other tertiles, $P < 0.08$.

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