Fluctuations in GAD_{65} Antibodies After Clinical Diagnosis of IDDM in Young Children

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OBJECTIVE — To investigate whether the presence of GAD antibodies at onset of IDDM correlates to a more aggressive rate of β-cell destruction after clinical onset.

RESEARCH DESIGN AND METHODS — We studied GAD antibodies at onset of disease, after 1 year, and after 6 years in 33 consecutively referred children (mean age 8.08, range 1.7–16.3). In a subset of 11 patients, GAD antibodies were studied very frequently. The correlation between GAD antibodies and clinical parameters, including glycosylated hemoglobin, residual insulin secretion, and insulin dosage, was evaluated.

RESULTS — GAD antibody titers were highly variable. Four patients became GAD antibody positive weeks to years after clinical onset. Other patients switched between testing positive and negative for GAD antibodies shortly after clinical onset. No correlation was found between the presence of GAD antibodies and the rate of β-cell destruction, but patients with high GAD antibody indexes at onset had significantly higher glycosylated hemoglobin levels.

CONCLUSIONS — GAD antibodies at clinical onset do not predict the rate of β-cell destruction in young children with newly diagnosed IDDM. The highly variable GAD antibody levels suggest variation of the autoimmune process.

Antibodies against GAD are detected in ~80% of newly diagnosed patients with IDDM and prediabetic individuals (1,2). Petersen and colleagues (3) showed that the presence of GAD antibodies at clinical onset of IDDM predicted the course of β-cell destruction in adolescent and adult patients. However, β-cell destruction in young children occurs faster, and data on prospective follow-up of GAD antibodies in IDDM in newly diagnosed young children are scarce. We examined the course of GAD antibodies and their correlation with the clinical course of the disease in 33 consecutively referred children with newly diagnosed IDDM.

RESEARCH DESIGN AND METHODS

— Thirty-three consecutively referred newly diagnosed children (15 girls, mean age at diagnosis of IDDM 8.08 ± 3.97 [range: 1.7–16.3]), who entered a trial of continuous subcutaneous insulin infusion (CSII) in 1982–1984 (4,5), were studied. Serum samples and clinical data were collected over a 2-year period. Serum samples were available up to a maximum of 8 years of diabetes duration (mean 6 years, range 3–8, due to leaving clinic and difficulties in obtaining new samples). The study was approved by local ethics committees in accordance with the Declaration of Helsinki, and signed informed consent was obtained.

GAD antibodies were tested by immunoprecipitations of BHK (baby hamster kidney) cells which stably expressed human GAD_{65}, followed by SDS-PAGE and fluorography (2,3) The results were correlated to standard negative and positive control sera and expressed as GAD index. Dilution curves showed that this gel method is at least one dilution step more sensitive due to low background levels and unambiguous identification of the GAD doublet band (data not shown). This assay detected 84% GAD positives (n = 150) compared with 80% for the widely applied radioimmunoassay (RIA). GAD antibodies were tested in sera collected at onset, after 1 year, and after 6 years (mean, range 3–8 years) of IDDM duration in all 33 children. The course of GAD antibodies was studied in detail in a subset of 11 children, of whom 12–15 serum samples from the first 2 years of disease were available. In the immunoprecipitations, the interassay variation for the positive control was 12%. A GAD index of 0.16 or lower was negative. This was confirmed by 3 months exposure of gels to X-ray film. Technical disturbances by prozone effects or concentration effects were excluded by dilution experiments and by normal sodium, potassium and total protein levels in sera with high GAD antibody titers (data not shown). All sera were tested at least three times in independent experiments. The endogenous insulin production (24-h urinary C-peptide secretion) and total glycosylated hemoglobin were measured as described (5–7).

Complete data sets were available in 31 patients. Data were analyzed combined and separately for the conventional and the CSII group. For comparisons between groups, Wilcoxon's rank-sum test (SPSS-PC+, SPSS Inc., Chicago, IL) was used. The correlation between GAD antibodies and clinical parameters was tested by regression analysis. Age-corrected C-peptide secretion (ccpep) and GAD indexes were log-transformed to obtain Gaussian-shaped distribution (lnccpep = ln[ccpep + 1] and lnGAD − index = ln[GAD − index + 0.1]).

RESULTS — At onset, 23 (70%) of the

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Received for publication 29 April 1996 and accepted in revised form 1 November 1996.

ccpep, C-peptide secretion; CSII, continuous subcutaneous insulin infusion; RIA, radioimmunoassay.

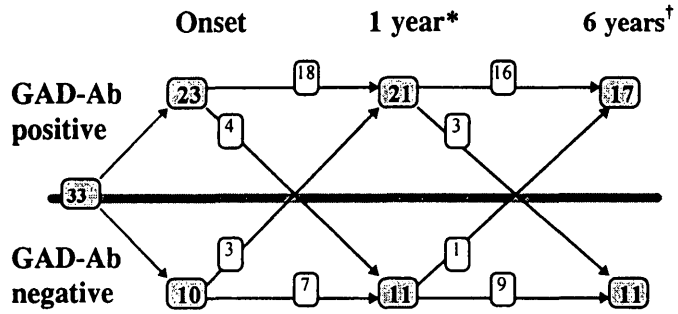

Figure 1—*The course of GAD antibodies positivity/negativity of 33 children with IDDM at onset and after 1 and 6 years of disease duration. Shaded numbers indicate numbers of patients; unshaded numbers indicate numbers of seroconverting patients. *Serum from one patient not available. †Sera from four patients not available for testing.*

children were GAD antibody positive, 10 (30%) patients were negative. The GAD status was independent of age of onset. Figure 1 depicts the variation in the GAD status of the children at onset and after 1 and 6 years. Seventeen of 28 children (59%) tested GAD antibody positive at longer duration of diabetes (mean 6 years). In three of 11 patients tested repeatedly during the first 2 years of disease, we observed a switch from negative to positive (and back) within weeks to months, whereas in other patients high peaks in GAD index were followed by lower titers in consecutive sera tested. One patient converted from GAD antibody negative to weakly positive and back within a month. Another was negative for GAD antibodies at onset of the disease, but positive at 1–2 months and 4–5 months duration, remaining negative during the intervening period. Strikingly, one patient tested negative for GAD antibodies in all serum samples taken during the first 16 months of disease and tested positive in the next serum sample, taken at 56 months of disease duration. In Fig. 2, fluctuations in GAD antibody indexes in the repeatedly tested patients are shown graphically. A significant correlation between the glycosylated hemoglobin at onset and GAD index at onset, but not with GAD status at onset, was found ($R = 0.38$, $P = 0.037$). No other correlations between GAD status or GAD index and clinical parameters (Table 1) at any timepoint were observed. The CSII-treated patients experienced a longer remission period (defined as three successive age-corrected C-peptide measurements of 30% or less) than did the conventional treated patients (8). However, this was not reflected by GAD antibodies.

CONCLUSIONS — Despite intense efforts, blood glucose regulation in young children with IDDM remains unsatisfactory. Endogenous insulin production may facilitate blood glucose regulation. Better understanding of the heterogeneous disease process, also during the honeymoon, may be beneficial for later control. We studied whether the presence of GAD antibodies at clinical onset in young children is correlated to a more aggressive β-cell destruction, as was suggested in adolescents and adults (3). No such correlation was found. In fact, the observed correlation between glycosylated hemoglobin levels and GAD antibodies suggests a less aggressive β-cell autoimmunity in GAD antibody–positive patients, allowing for a longer preclinical period with deranged blood glucose metabolism. Whereas others previously described the yet unexplained phenomenon of persisting GAD antibodies long after clinical onset of IDDM, we identified highly fluctuating titers. The previously described heterogeneity of autoimmune destruction in the human pancreas (9) and functional heterogeneity of β-cells, which may result in different levels of GAD_{65} expression and blood glucose regulation (10), may account for the observed fluctuations. Excluding technical failures, it is conceivable that similar fluctuations exist in the preclinical phase as has been found for ICAs (11,12). However, in these studies once positive samples did not become negative. Our data warrant repetitive testing of GAD antibodies in IDDM prediction.

We prefer the traditional gel method for delicate analyses. RIA, widely used in prediction studies, has the disadvantage of relatively high background levels with concomitant problems of threshold setting. The gel method has a low background and can identify unambiguously the GAD doublet. Moreover, titrations show a higher sensitivity of this method. Low titers in the RIA might be underestimated, in particular when indexes are used to compare different experiments (M.R.B., J.S. Petersen, A. Van Driel, C. Van Donselaar, G.J.B., T. Dyrberg, H.-J.A., unpublished observations).

We conclude that GAD antibodies are not prognostic for the rate of β-cell destruction or duration of the honeymoon phase in this group of young children. The observed qualitative and quantitative variability in GAD antibodies might explain the lack of correlation with clinical parameters. GAD antibodies are present in 70% of the patients at clinical onset, but in four out of 33 children (12%) GAD antibodies appeared weeks to months after diagnosis. If the observed fluctuations in GAD anti-

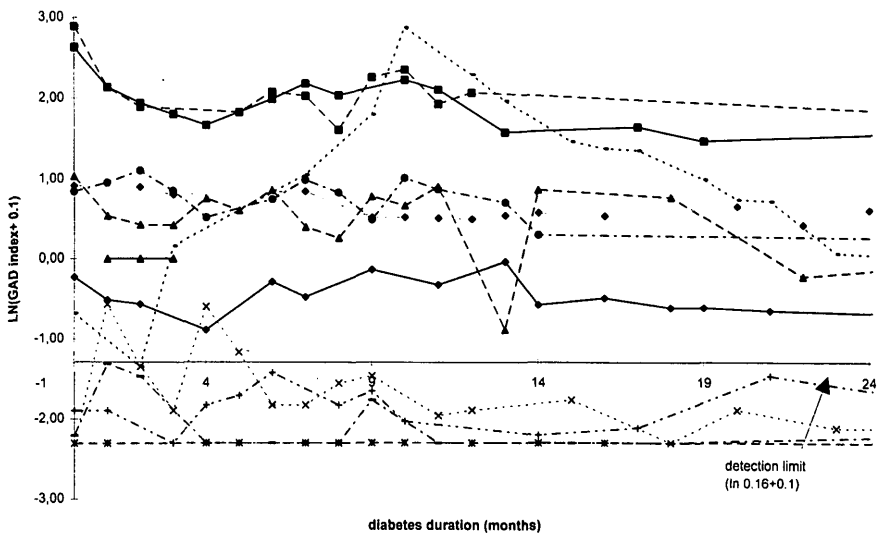

Figure 2—*The course of GAD indexes in 11 young patients during the initial 2 years of disease.*

Table 1—*Correlation coefficient of GAD indexes at onset with clinical parameters at onset and later in disease*

	Age-corrected C-peptide			GHb			Insulin dosage		
	Onset	3 months	6 months	Onset	3 months	6 months	Onset	3 months	6 months
All	−0.13 NS			0.38 $P = 0.037$			0.11 NS		
CSII		0.53 NS	0.21 NS		0.15 NS	−0.10 NS		0.16 NS	−0.06 NS
Conventional		−0.03 NS	−0.09 NS		−0.53 NS	0.32 NS		−0.30 NS	0.07 NS

bodies occur in the prediabetic phase as well, this has implications for diabetes prediction.

Acknowledgments— The study was supported by the Child Health and Well-Being Fund, the Diabetes Fonds Nederland (DFN92604 and DFN/NWO 900-570-005). M.P. was supported by an Erasmus University student exchange program.

We thank the patients and their parents for their cooperation and Dr. J.L. Molenaar for analyzing the ICAs.

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