Rapid Publications

Antibodies to Glutamic Acid Decarboxylase Reveal Latent Autoimmune Diabetes Mellitus in Adults With a Non–Insulin-Dependent Onset of Disease

TIINAMAIJA TUOMI, LEIF C. GROOP, PAUL Z. ZIMMET, MERRILL J. ROWLEY, WILLIAM KNOWLES, AND IAN R. MACKAY

The classification of adults with diabetes mellitus can be invalidated by patients who initially present as NIDDM but who later become frankly insulin dependent. In some of these, the pathogenesis could be similar to that in IDDM, namely autoimmune destruction of the pancreatic β-cells. We studied 102 patients >35 yr of age at diabetes onset who had initially been nonketotic and non-insulin-dependent for ≥6 mo. They were classified according to glucagon-stimulated C-peptide levels into an insulin-deficient group (n = 33) and a non–insulin-deficient group (n = 69). We measured antibodies to GAD, islet cell cytoplasm, thyroid antigens, and gastric parietal cells in both groups. Anti-GAD was significantly higher in the insulin deficient group, 76% (25 of 33), than in the non–insulin deficient group, 12% (8 of 69), and this difference was substantially greater than that shown for ICAs. Thus, in a proportion of adults who present with NIDDM, a slowly evolving autoimmune insulitis can be revealed by testing for anti-GAD. This could have important connotations not only for early intervention, but also for the correct classification of diabetes. *Diabetes* 42:359–62, 1993

From the Centre for Molecular Biology and Medicine, Monash University, Clayton, Victoria, Australia; the Fourth Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland; The International Diabetes Institute, Caulfield, Victoria, Australia; and Miles Research Laboratories, West Haven, Connecticut.

Address correspondence and reprint requests to Dr. Ian R. Mackay, Centre for Molecular Biology and Medicine, Monash University, Clayton, 3168 Victoria, Australia.

Received for publication 2 November 1992 and accepted 5 November 1992.

GAD, glutamic acid decarboxylase; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; ICA, islet cell antibody; CF-ICA, complement-fixing islet cell antibody; JDF, Juvenile Diabetes Foundation; TMA, thyroid microsomal antibodies; TgA, thyroglobulin antibodies; GPCA, gastric parietal cell antibody; CV, coefficient of variation; BMI, body mass index; df, degrees of freedom; LADA, latent autoimmune diabetes of adults.

In patients with adult-onset diabetes mellitus, the classification into IDDM or NIDDM categories may be difficult (1). It is estimated that, among patients with IDDM, 40% develop diabetes by 15 yr of age, 30% between 15 and 34 yr of age, and 30% thereafter. Also, annually, 1–2% with apparent NIDDM become insulin deficient (2). Most patients with NIDDM who eventually receive insulin have a relative rather than an absolute deficiency of insulin in that they cannot increase insulin secretion to compensate for their degree of insulin resistance (3). However, certain NIDDM patients develop an absolute insulin deficiency within a few years, the so-called type 1 1/2 diabetes (4) or latent IDDM (5). These patients have many features of classical IDDM: low C-peptide levels, low body-weight, ICAs, other organ-specific autoantibodies, and a high frequency of HLA DR3 and DR4 (5–9). However, none of these features conclusively distinguishes between IDDM and NIDDM.

In this study on initially nonketotic NIDDM of adult onset, we investigated the frequency of autoantibodies to GAD (10), formerly the 64,000-M_r islet cell antigen. Anti-GAD correlated significantly with the insulin requirement of the patients.

RESEARCH DESIGN AND METHODS

The study group of 102 patients was selected from the register of the Helsinki Diabetic Association and the outpatient clinic of the Helsinki University Hospital in 1984–1988 (5,9) with these entry criteria: >35 yr of age at diagnosis, nonketotic diabetes without insulin treatment over at least 6 mo of observation, and an initial diagnosis of NIDDM. Patients with secondary diabetes, with other diseases, or using drugs known to influence glucose metabolism were excluded. All patients were examined by one of us and questioned for the occurrence of diseases, including diabetes mellitus, NIDDM,

TABLE 1
Clinical characteristics of patients studied with nonketotic onset of diabetes after 35 yr of age in relation to their requirement of insulin and positivity for antibodies to GAD

	Patient groups			
	Insulin deficient	Non–insulin deficient	Anti-GAD$^+$	Anti-GAD$^-$
n	33	69	33	69
Sex (M/F)	19/14	35/34	20/13	34/35
Age (yr)	55.7 ± 7.8	57.4 ± 7.7	56.8 ± 7.5	57.0 ± 7.9
Age at onset of diabetes (yr)	50.6 ± 8.6	49.4 ± 7.8	50.0 ± 7.7	49.1 ± 7.7
(range)	(38–64)	(35–65)	(35–64)	(35–62)
Duration of diabetes (yr)	6.1 ± 5.3	9.7 ± 10.4	6.5 ± 5.8	8.6 ± 5.0
(range)	(0.5–20)	(1–24)	(1–20)	(0.5–24)
NIDDM in first-degree relatives (%)	49 (16/33)	61 (42/69)	49 (16/33)	61 (42/69)
IDDM in first-degree relatives (%)	9 (3/33)	7 (5/69)	12 (4/33)	6 (4/69)
BMI (kg/m^2)	24.7 ± 3.7	27.5 ± 3.5	24.0 ± 5.7	27.0 ± 4.9
(% >25 kg/m^2)*	(36)	(71)	(39)	(70)
HbA_1 (%)	10.5 ± 2.0	9.7 ± 3.3	9.8 ± 2.2	9.8 ± 2.0
Postglucagon C-peptide (nM)	0.29 ± 0.18	1.1 ± 0.35	0.43 ± 0.41	1.0 ± 0.40

Data are means ± SD.
*$\chi^2 = 12.6$, $P < 0.001$ for insulin deficient vs. non–insulin deficient; $\chi^2 = 9.8$, $P < 0.005$ for anti-GAD$^+$ vs. anti-GAD$^-$.

or IDDM, in first-degree relatives. Frozen serum samples that had been obtained 3.2 ± 2.0 yr (mean ± SD) after the initial assessment were used for the anti-GAD assays.

Metabolic studies. β-cell function was assessed after an overnight fast by measuring C-peptide before and 6 min after an intravenous injection of 1 mg of glucagon, with oral hypoglycemic drugs withheld 24 h beforehand. Glucagon-stimulated C-peptide values <0.60 nM specified insulin deficient, whereas higher C-peptide levels specified non–insulin deficient, whether or not patients were receiving insulin. There were 33 patients in the insulin deficient and 69 in the non–insulin deficient category, although insulin therapy had been started for 57 patients by 1988.

Serological tests for ICA and thyrogastric autoantibodies. ICA, and CF-ICA, were determined by indirect immunofluorescence following a protocol adopted in the first international workshop on the standardization of cytoplasmic ICAs (11,12) before the international JDF standard was introduced. TMA and TgA were measured by passive hemagglutination with commercial kits (Microsome Test, Fujizoki, Tokyo). GPCAs were detected by indirect immunofluorescence on cryostat sections of mouse gastric mucosa. Titers exceeding 1:100 for TMA, 1:25 for TgA, and 1:10 for GPCA were considered positive. The 60 healthy control sera (33 women, 27 men; matched for age) that were studied concurrently gave a frequency of positive results of 0% for ICA, 20% for TMA, 2% for TgA, and 7% for GPCA (9).

Autoantibodies to GAD. These were measured by a radioimmunoprecipitation assay (13). Briefly, affinity-purified porcine brain GAD, which was enzymatically active and contained both known isoforms (67,000 and 65,000 M_r), was iodinated with ^{125}I. After preabsorption with pooled normal sera, 100,000 cpm of [^{125}I]GAD was incubated overnight with test sera diluted 1:2 in Tris-Triton buffer (0.02 M Tris, 0.15 M NaCl, 0.5% w/v Triton X-100, pH 7.4). [^{125}I]GAD-antibody complexes were precipitated by protein A-Sepharose and counted for radioactivity after three washes. The results were expressed as units equalling the percentage of radioactivity precipitated by the test sera of that precipitated by the reference serum, which thus had 100 U. The interassay CV was 7% for another high-positive control (mean 120 U) and 14% for a low-positive control (mean 33 U). The intra-assay variation between duplicates was 8% for the low-positive control. The normal range of reactivity was defined by using 53 Australian blood donor sera tested concurrently. The mean was 8.2 ± 2.7 U (range 4–18 U). The limit for positivity was set at 18 U, which exceeds the mean ± 3 SD for the controls.

Statistical analysis. The χ^2 test with Yates' correction was used to determine statistical significances of differences between group frequencies, and unpaired Student's *t* test to compare means of anti-GAD levels, where appropriate.

RESULTS

The glucagon-stimulated C-peptide levels were <0.60 nM in 33 patients (insulin deficient group), and >0.60 nM in 69 patients (non–insulin deficient group). The mean age, age at onset of diabetes, duration of the disease, and HbA_1 levels were similar in both groups (Table 1). The BMI was lower for the insulin deficient patients than for the non–insulin deficient patients, being >25 kg/m^2 in 36% of the former and 71% of the latter ($\chi^2 = 12.6$, $P < 0.001$) (Table 1). Antibodies to GAD were detected in most of the insulin deficient patients, 25 of 33 (76%), in contrast to 8 of 69 (12%) of the non–insulin deficient patients ($\chi^2 = 37.7$, $P < 0.001$) (Fig. 1). No significant difference was observed in the anti-GAD positivity rate between men and women (men: 9 of 14 insulin deficient, 4 of 34 non–insulin deficient; women 16 of 19 insulin deficient, 4 of 34 non–insulin deficient) nor in their levels of anti-GAD (data not shown). Among those positive for anti-GAD, the range of values was similar for the insulin deficient and non–insulin deficient groups, and although the mean levels were higher for the insulin deficient group, the difference was not statistically significant

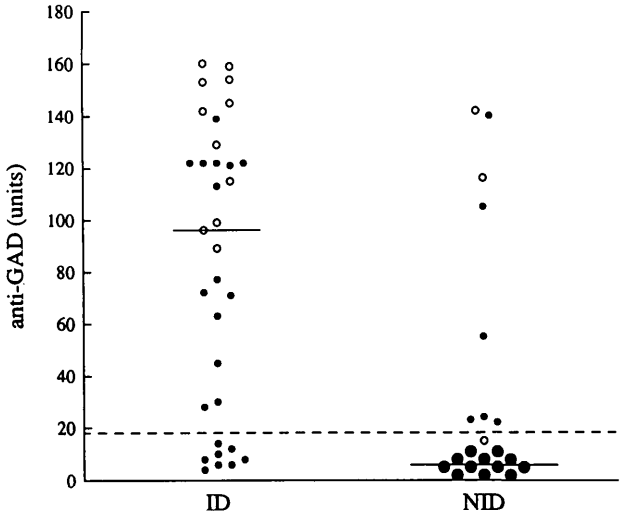

FIG. 1. Levels of antibodies to GAD (anti-GAD) in 33 insulin deficient (ID) and 69 non-insulin deficient (NID) patients. The results of the radioimmunoprecipitation assay are expressed in units (see METHODS). (- - - -), the upper normal limit for positivity (18 U); (——), medians for the two groups. (•), single cases; (●), a group of 5 cases; (○), cases positive for ICA and CF-ICA.

(108 ± 39 vs. 78 ± 53 U, t[df 31] = 1.7, $P < 0.1$) (Fig. 1).

The age distribution, age at onset of diabetes, duration of disease, and levels of HbA_1 were similar for patients anti-GAD$^+$ or anti-GAD$^-$ (Table 1). Differences in BMI and the family history of NIDDM similar to those between insulin deficient and non-insulin deficient groups were found between anti-GAD$^+$ and anti-GAD$^-$ patients (Table 1). Among the ID patients, no clinical characteristics distinguished the 8 anti-GAD$^-$ from the 25 anti-GAD$^+$ patients. Data on ICA tests, performed some 3 yr earlier (5,9), were available for 29 of the anti-GAD$^+$ patients and all 69 of the anti-GAD$^-$ patients (Table 2, Fig. 1). ICAs were detected in 18 patients, and CF-ICA in 14 of these. All but one of the CF-ICA$^+$ patients had antibodies to GAD; they comprised 45% of all of the anti-GAD$^+$ cases. Five of 69 (7%) anti-GAD$^-$ patients had been positive for ICA, and 1 also for CF-ICA. However, 4 of these, including 1 with CF-ICA had normal C-peptide levels (basal 0.38–0.74 nM, postglucagon 0.84–1.32 nM), and high BMI (27.2–36.2 kg/m^2) 4–9 yr after the diagnosis of diabetes mellitus, suggestive of true NIDDM. The fifth fitted the clinical diagnosis of latent IDDM because she was diagnosed 7 yr earlier and treated with insulin for the last 2 yr, had minimal insulin production (glucagon-stimulated C-peptide 0.09 nM), a normal BMI (25.1 kg/m^2), and positive tests for TMA and GPCA.

TABLE 2
Frequency of ICAs including CF-ICA and thyrogastric antibodies in patients with adult-onset diabetes in relation to anti-GAD and requirement for insulin

	Anti-GAD$^+$ patients	Anti-GAD$^-$ patients
n	33	69
ICA (%)	45 (13/29)	7 (5/69)*
Insulin deficient group	52 (11/21)	13 (1/8)
Non-insulin deficient group	25 (2/8)	7 (4/61)
CF-ICA (%)	45 (13/29)	1 (1/69)†
Insulin deficient group	52 (11/21)	0 (0/8)
Non-insulin deficient group	25 (2/8)	2 (1/61)
TMA (%)	28 (8/29)	19 (12/65)
TgA (%)	7 (2/29)	3 (2/65)
GPCA (%)	42 (8/19)	10 (3/31)‡

Comparisons between anti-GAD$^+$ and anti-GAD$^-$ groups.
*$\chi^2 = 16.8$, $P < 0.001$.
†$\chi^2 = 27.9$, $P < 0.001$.
‡$\chi^2 = 4.6$, $P < 0.05$.

DISCUSSION

The presence of ICA was previously shown to correlate with progression to insulin deficiency in a subgroup of adult patients who presented with NIDDM, according to both cross-sectional and prospective studies (5–9,14, 15). Compared with other patients with NIDDM who were given insulin, the ICA$^+$ patients tended to have a shorter duration of diabetes before beginning insulin treatment (5,6,14), a lower body weight, lower C-peptide levels, and a subsequent deterioration of β-cell function (5). The increased frequency of HLA DR3 and DR4 (9,14) and organ-specific autoantibodies (5,6) further strengthen the similarity to IDDM.

In this cross-sectional and partly retrospective study of cases of nonketotic diabetes that presented after 35 yr of age and were diagnosed as NIDDM, a clear relationship was found between the presence of anti-GAD and insulin deficiency. Because hyperglycemia per se is currently a sufficient indication to start insulin in NIDDM, postglucagon C-peptide levels were used to confirm insulin deficiency rather than the mode of treatment that had been adopted. By this criterion, ~33% of those studied (33 of 102) were insulin deficient, whereas ~50% (57 of 102) were actually treated with insulin. Some 36% of the insulin deficient group, and 71% of the non-insulin deficient group, had BMI >25 kg/m^2 ($P < 0.001$): these frequencies are remarkably similar to those described for Finnish IDDM (37%) and NIDDM (77%) patients of all ages (16). Moreover, the insulin deficient and non-insulin deficient groups did not differ by sex distribution, age, age at onset, duration, glycemic control, or family history of either IDDM or NIDDM. On the other hand, 76% of the 33 insulin deficient patients were anti-GAD$^+$ in contrast to only 12% of the 69 non-insulin deficient patients ($P < 0.001$). Thus the anti-GAD assay had a sensitivity of 76% and a specificity of 88% in differentiating between the insulin deficient and non-insulin deficient patients. Note that 3 of 8 non-insulin deficient patients that were anti-GAD$^+$ had levels that barely exceeded the upper limit of normal, whereas the other 5 had levels indistinguishable from those in the insulin deficient group. Of note, 6 of these 8 patients had required insulin therapy because of worsening glycemic control. The possibility that their β-cell function subsequently deteriorated could not be excluded, because C-peptide measurements were not repeated on patients already under insulin treatment.

Our results clearly show that testing for anti-GAD was superior to the traditional ICA testing in distinguishing the

insulin deficient from the non–insulin deficient patients. Note that these two tests were done at different times with the ICA tests being done some years earlier and, in most cases, either before or within a year of starting insulin. Thus, the difference in time of testing should favor ICA, yet testing for anti-GAD was still more informative even though performed years later. Although prospective studies on anti-GAD are few, the antibodies are demonstrable in most IDDM patients both before and at the onset of the disease (13,17–19), persist for at least 3 yr after diabetes onset (20), and are detectable even 10–20 yr after diagnosis (13), but with some decline in time in their frequency. Thus, according to data from our laboratory, antibodies to purified porcine brain GAD are present in 69% of adults with IDDM for <1 yr, and in 59% of those with IDDM for >3 yr (13) and, similarly, in 75 and 60%, respectively, of children with IDDM of recent onset or longer duration (Q-Y Chen, S.W. Serjeantson, T.T., M.J.R., I.R.M., P.Z.Z., unpublished observations). This study shows, in patients who had initially presented with NIDDM, that the strong correlation between anti-GAD and insulin deficiency is demonstrable several years after diagnosis.

The anti-GAD$^+$ patients overlapped closely with the insulin deficient group, so that the metabolic characteristics of patients anti-GAD$^+$ or anti-GAD$^-$ were comparable with those of the insulin deficient and NID patients, respectively. Thus positivity for antibodies to GAD was associated with insulin deficiency and lower body mass. The frequency of thyroid and gastric autoantibodies was increased in the anti-GAD$^+$ patients, suggestive of an increased frequency among these of polyendocrine autoimmunity.

The frequent finding of antibodies to GAD specifies a syndrome of LADA, and recognition of this has clear clinical implications. First, it further confirms that patients with LADA represent a discrete subgroup of IDDM with a pathogenesis similar to that of IDDM. Second, identification of this group at presentation would allow its subsequent exclusion in studies directed towards the pathogenesis of NIDDM. Third, given their strong probability of developing frank insulin deficiency, these patients should be followed more carefully to ensure prompt institution of insulin treatment, which may reduce various short- or long-term complications of diabetes, or even early treatment with immunosuppressive agents to halt the autoimmune process. Several studies already in progress are aimed at the prevention of diabetes in high-risk groups, e.g., ICA$^+$ siblings of IDDM patients. It would seem more appropriate to assess initially such treatment in LADA to determine whether residual β-cell function can be preserved.

ACKNOWLEDGMENTS

The work has been supported by a grant from the Miles Diagnostics, and also by a grant to T.T. from the Cultural Foundation of Finland.

The tests for ICA were provided by Professor Gian Franco Bottazzo, Department of Immunology, Middlesex Hospital Medical School, London, United Kingdom, and for thyrogastric autoantibodies by Dr. Aaro Miettinen, Department of Bacteriology and Immunology, University of Helsinki, Helsinki, Finland.

REFERENCES

1. Harris M, Zimmet P: Classification of diabetes mellitus and other categories of glucose intolerance. In *International Textbook of Diabetes Mellitus*. Alberti KGMM, DeFronzo RA, Keen H, Zimmet P, Eds. Wiley, 1992, p. 3–18
2. Hagopian W, Lernmark Å: Autoimmune diabetes mellitus. In *The Autoimmune Diseases* II. Rose NE, Mackay IR, Eds. Academic Press, 1992, p. 235–78
3. Porte D Jr: β-cells in type II diabetes mellitus. *Diabetes* 40:166–80, 1991
4. Editorial: Insulin-dependent? *Lancet* 2:809–10, 1985
5. Groop LC, Bottazzo GF, Doniach D: Islet cell antibodies identify latent type I diabetes in patients aged 35–75 years at diagnosis. *Diabetes* 35:237–41, 1986
6. Irvine WJ, Gray RS, McCallum CJ, Duncan LJP: Clinical and pathogenic significance of pancreatic-islet-cell antibodies in diabetes treated with oral hypoglycaemic agents. *Lancet* 1:1025–27, 1977
7. Irvine WJ, Sawers JSA, Feek CM, Prescott RJ, Duncan LJP: The value of islet cell antibody in predicting secondary failure of oral hypoglycaemic agent therapy in diabetes mellitus. *J Clin Lab Immunol* 2:23–26, 1979
8. Di Mario U, Irvine WJ, Borsey DQ, Kyner JL, Weston J, Galfo C: Immune abnormalities in diabetic patients not requiring insulin at diagnosis. *Diabetologia* 25:392–95, 1983
9. Groop LC, Miettinen A, Groop P, Meri S, Koskimies S, Bottazzo GF: Organ-specific autoimmunity and HLA-DR antigens as markers for β-cell destruction in patients with type II diabetes. *Diabetes* 37:99–103, 1988
10. Baekkeskov S, Aanstoot H, Christgau S, Reetz A, Solimena M, Cascalho M, Folli F, Richter-Olesen H, De Camilli P: Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* (Lond) 347:151–56, 1990
11. Bottazzo GF, Dean BM, Gorsuch AN, Cudworth AG, Doniach D: Complement-fixing islet cell antibodies in type I diabetes: possible monitors of active β-cell damage. *Lancet* 1:668–72, 1980
12. Bottazzo GF, Gleichmann H: Immunology and Diabetes Workshops: report of the first international workshop on the standardization of cytoplasmic islet cell antibodies. *Diabetologia* 29:125–26, 1986
13. Rowley MJ, Mackay IR, Chen Q, Knowles WJ, Zimmet PZ: Antibodies to glutamic acid decarboxylase discriminate major types of diabetes mellitus. *Diabetes* 41:548–61, 1992
14. Gleichmann H, Zörcher B, Greulich B, Gries FA, Henrichs HR, Bertrams J, Kolb H: Correlation of islet cell antibodies and HLA-DR phenotypes with diabetes mellitus in adults. *Diabetologia* 27:90–92, 1984
15. Landin-Olsson M, Karlsson FA, Lernmark Å, Sundkvist G, the Diabetes Incidence Study in Sweden Group: Islet cell and thyrogastric antibodies in 633 consecutive 15- to 34-yr-old patients in the diabetes incidence study in Sweden. *Diabetes* 41:1022–27, 1992
16. Eriksson J, Forsén B, Häggblom M, Teppo A-M, Groop L: Clinical and metabolic characteristics of Type 1 and Type 2 diabetes: an epidemiological study from the Närpes community in Western Finland. *Diabetic Med* 9:654–60, 1992
17. Baekkeskov S, Landin M, Kristensen JK, Srikanta S, Bruining GJ, Mandrup-Poulsen T, de Beaufort C, Soeldner JS, Eisenbarth G, Lindgren F, Sundquist G, Lernmark Å: Antibodies to a 64,000 M_r human islet cell antigen precede the clinical onset of insulin-dependent diabetes. *J Clin Invest* 79:926–34, 1987
18. Atkinson MA, Maclaren NK, Scharp DW, Lacy PE, Riley WJ: 64,000 M_r autoantibodies as predictors of insulin-dependent diabetes. *Lancet* 335:1357–60, 1990
19. Kaufman DL, Erlander MG, Clare-Salzler M, Atkinson MA, Maclaren NK, Tobin AJ: Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. *J Clin Invest* 89:283–92, 1992
20. Christie MR, Daneman D, Champagne P, Delovitch TL: Persistence of serum antibodies to 64,000-M_r islet cell protein after onset of type I diabetes. *Diabetes* 39:653–56, 1990