Clinical, Autoimmune, and Genetic Characteristics of Adult-Onset Diabetic Patients With GAD Autoantibodies in Japan (Ehime Study)

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OBJECTIVE — To characterize the clinical, autoimmune, and genetic features in Japanese adult-onset diabetic patients with GAD autoantibodies.

RESEARCH DESIGN AND METHODS — GAD autoantibodies (GADab) were screened in 4,980 diabetic patients with age of onset >20 years in the hospital-based Ehime Study, and the GADab-positive ($GADab^+$) patients were then divided into two groups according to their insulin secretion and compared with nondiabetic subjects. The insulin-deficient state was defined as <0.33 nmol/l serum C-peptide (CPR) at 2 h postprandial or 6 min after a 1-mg glucagon load.

RESULTS — GADab was detected in 188 (3.8%) of the 4,980 diabetic patients tested. Of these patients, 72 (38.3%) were classified as insulin deficient, 97 (51.6%) were classified as non–insulin deficient, and 19 (10.1%) were unclassified. The $GADab^+$ insulin-deficient patients were characterized by young age at onset of diabetes, low BMI, low maximum BMI, and high levels of HbA_{1c}. The prevalence of IA-2 autoantibodies and thyrogastric autoantibodies in the $GADab^+$ insulin-deficient patients were significantly higher than those in the $GADab^+$ non–insulin-deficient patients ($P < 0.05$). $GADab^+$ patients with insulin deficiency had increased frequencies of HLA DRB1*0405-DQB1*0401, *0802-*0302, and *0901-*0303 haplotypes, whereas the frequency of only HLA DRB1*0405-DQB1*0401 was increased in the case of $GADab^+$ non–insulin-deficient patients. Of note is the fact that the $GADab^+$ non–insulin-deficient group did not differ from healthy control subjects with respect to type 1 diabetes protective haplotype HLA DRB1*1502-DQB1*0601. A total of 13% of the $GADab^+$ patients with diabetes had genotypes comprising the DRB1*1501-DQB1*0602 or *1502-*0601 and were characterized by old age at onset of diabetes, high BMI, resistance to the insulin-deficient state, low titer of GADab, and low frequency of other organ-specific autoantibodies.

CONCLUSIONS — We conclude that $GADab^+$ non–insulin-deficient patients differ from $GADab^+$ patients with insulin deficiency with respect to clinical characteristics, humoral autoimmunity to other organ-specific autoantibodies, as well as HLA class II genes.

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Although type 1 diabetes is characterized by the progressive autoimmune destruction of islet β-cells, heterogeneous mechanisms and different clinical courses have been reported (1,2). Most commonly, type 1 diabetes is observed in children and is characterized by rapid progression to insulin dependency. However, it is well known that the clinical onset of type 1 diabetes is not confined to childhood. Epidemiological data suggest that 30–50% of the cases may develop clinical signs of type 1 diabetes after 20 years of age (3). The revised World Health Organization and American Diabetes Association classification of diabetes encompasses clinical stages as well as etiological types of diabetes (4,5). Thus, the presence of anti-islet autoantibodies in diabetic patients in non–insulin-deficient stages makes it likely that these individuals have the type 1 autoimmune process. Some Japanese patients are characterized by persistent islet cell antibody (ICA) positivity and a slowly progressive deterioration of β-cell function through the non–insulin-dependent state and ultimately to an insulin-dependent state for several years (6). This subset of type 1 diabetes is referred as the slowly progressive form of type 1 diabetes (SPIDDM), latent autoim-

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Abbreviations: CPR, C-peptide; CV, coefficient of variation; GADab, GAD autoantibodies; $GADab^+$, GADab-positive; ICA, islet cell antibody; LADA, latent autoimmune diabetes in adults; RIA, radioimmunoassay; SPIDDM, slowly progressive form of type 1 diabetes; TG, thyroglobulin; TPO, thyroid peroxidase.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

mune diabetes in adults (LADA), or type 1.5 diabetes (6–8).

ICA and autoantibodies to GAD (GADab) are characteristic of the immune-mediated form of type 1 diabetes and are present in 70–80% of patients with new-onset type 1 diabetes (9,10). Recently, several studies have suggested that GADab might be useful as a predictive marker for the development of insulin deficiency in type 2 diabetes in Caucasians as well as in Japanese (8,11–13). The U.K. Prospective Diabetes Study reported that, among patients with type 2 diabetes, the frequency of GADab ranged from 34% of those aged 25–35 years at diagnosis to 7% of those aged 55–65 years at diagnosis. Of the patients with both ICA and GADab, 94% required insulin therapy versus only 14% of those without either autoantibody. These patients with insulin-requiring type 2 diabetes and GADab may be classified as SPIDDM or LADA (12). However, the issue of whether type 2 diabetes (non–insulin-deficient diabetes) with GADab share a genetic background with type 1 diabetes remains to be solved.

This study was conducted to clarify the clinical, autoimmune, and genetic characteristics of adult GADab-positive (GADab$^+$) Japanese diabetic patients in non–insulin-deficient state and to compare these characteristics with those of GADab$^+$ patients in insulin-deficient stages.

RESEARCH DESIGN AND METHODS

Subjects

GADab was screened in hospital-based samples from 4,980 adult-onset (>20 years) patients with diabetes who were patients in diabetic clinics of major hospitals in the Ehime area of Shikoku Island in Japan as a part of the Ehime Study during 1998 and 1999. The patients included both those being treated for diabetes and those with newly diagnosed diabetes, but other specific patients in whom underlying defects of the disease process could be identified were excluded. A total of 190 healthy subjects (128 men and 62 women) with a normal glucose tolerance, as assessed by a 75-g oral glucose tolerance test, with an absence of diabetes within the first-degree relatives served as control subjects. Details of the clinical data regarding GADab$^+$ diabetic patients were obtained during the screening and are described in Table 1. Glucose tolerance was assessed according to revised World Health Organization and American Diabetes Association criteria (4,5). Informed consent was obtained from all diabetic patients and nondiabetic control subjects. Insulin-deficiency was assessed by postprandial or glucagon-stimulated C-peptide levels. The criterion for the insulin-deficient state was <0.33 nmol/l of serum C-peptide at 2 h postprandial or 6 min after a 1-mg glucagon load (11). The insulin-deficient group includes both those with typical type 1 diabetes (3) and SPIDDM (6). The non–insulin-deficient state was defined as the group having >0.33 nmol/l of serum C-peptide levels after the loading test. Patients for whom serum C-peptide data were not available were excluded from the following analyses as an unclassified group.

Table 1—*Clinical characteristics of diabetic patients with GADab and healthy control subjects*

	Insulin-deficient	Non–insulin-deficient	Healthy control subjects
n	65	93	190
Sex (M/F)	23/42	43/50	128/62
Age at examination (years)	52.1 ± 13.7*	62.1 ± 13.1	49.5 ± 7.7
Age at diabetes onset (years)	41.2 ± 13.8*	51.3 ± 12.3	
Duration of diabetes (years)	11.1 ± 7.3	10.8 ± 9.1	
Family history of diabetes	16/61 (26.2)	35/90 (38.9)	0 (0)
BMI at examination (kg/m^2)	21.2 ± 2.7†	22.8 ± 4.1	23.3 ± 2.9
Max BMI (kg/m^2)	24.0 ± 3.8‡	26.6 ± 4.2	
HbA_{1c} (%)	8.5 ± 2.4‡	7.2 ± 1.7	4.8 ± 0.3
Retinopathy (%)	28.3	30.0	
Nephropathy (%)	25.9	31.1	
Neuropathy (%)	30.8	40.0	
Treatment (Insulin/oral hypoglycemic agents/diet)	65/0/0	35/40/18	

Data are *n*, means ± SE, or *n* (%). Insulin deficiency includes both typical type 1 diabetes and slowly progressive type 1 diabetes as described in the text. Family history was assessed within first-degree relatives. *$P < 0.0001$; †$P < 0.005$; ‡$P < 0.0005$ versus non–insulin-deficient.

GADab assay

GADab was determined at sampling by means of a commercially available radioimmunoassay (RIA) kit using ^{125}I-labeled recombinant human GAD65 as a tracer reagent (Cosmic, Tokyo, Japan) (14). The levels of GADab were normalized to a standard curve obtained from serial dilutions of the positive reference serum (256 units/ml at a dilution of 1:60). The interassay coefficient of variation (CV) and the intra-assay CV were 2.7% and 2.7%, respectively. Sera were considered to be GADab$^+$ when the level was >1.5 units/ml. The detection limit of the assay was determined to be 1.3 units/ml. In the First Proficiency Test of Diabetes Autoantibody Standardization Programs organized by Immunology of Diabetes Society, this assay (Lab ID148) had 82% sensitivity and 92% specificity.

IA-2 autoantibody radioassay

Autoantibodies to IA-2 were determined by radioligand binding assays in a 96-well assay format, as described previously, with some modifications (14,15). In vitro translated ^{35}S-labeled IA-2 (20,000 cpm of trichloroacetic acid precipitable protein) was incubated with serum in duplicate at a 1:25 dilution overnight at 4°C. The reaction was then transferred into a MultiScreen-DV 96-well filtration plate (Millipore, Burlington, MA) with protein A-Sepharose 4FF (Pharmacia, Freburg, Germany) and incubated for 45 min at 4°C. After washing the plate with Tris-buffered saline with Tween, the plate was counted for radioactivity in a 96-well microplate scintillation counter (MLC-2001; ALOKA, Tokyo, Japan). The antibody levels were expressed as an index using the counts from a positive and negative control serum. The cutoff index for autoantibodies to IA-2 was an index of 0.018. The interassay CV and intra-assay CV were 4.0% ($n = 10$) and 1.2% ($n = 5$), respectively. In the First Proficiency Test of Diabetes Autoantibody Standardization Programs organized by Immunology of Diabetes Society, this assay (Lab

ID148) had 58% sensitivity and 100% specificity.

Determination of other organ-specific autoantibodies

Anti-thyroglobulin (TG) antibodies and anti-thyroid peroxidase (TPO) antibodies were measured using a commercial RIA kit, following the instructions provided by the manufacturer (RSR Limited, Cardiff, U.K.). The cutoff titer for TG antibodies and TPO antibodies was 0.3 and 0.2 units/ml, respectively. Anti–parietal cell antibodies and anti-pituitary antibodies were analyzed by an indirect immunofluorescent technique using mouse stomach and rat pituitary, respectively.

HLA genotyping

HLA-DRB1 was typed using a Dynal RELI SSO HLA-DRB Test (UPGRADE) Package Insert following the manufacturer's protocol (Dynal, Wirral, U.K.). In this system, exon 2 was amplified by PCR from each genomic DNA obtained from peripheral blood and was then hybridized to an array of 35 immobilized sequence-specific oligonucleotide probes. When the four-digit DRB1 type was not completely obvious by the hybridization patterns due to overlapping, PCR restriction fragment–length polymorphism was used for high-resolution typing (16). PCR was performed using DRB1 allele-specific probes based on the 11th international HLA workshop protocol. HLA-DQB1 was also typed by PCR restriction fragment–length polymorphism using the SMITEST HLA DNA typing system, as described previously (17).

C-peptide concentration

Serum CPR level was measured before and 2 h after a standard breakfast, following an overnight fast. The level of glucagon-stimulated serum CPR was measured before and 6 min after an intravenous injection of 1 mg glucagon. Serum levels of CPR were determined using a commercial RIA kit (Daiichi III C-peptide; Daiichi Pharmaceutical, Tokyo, Japan), which was sensitive to levels ranging from 0.03 to 9.93 nmol/l.

Statistical analysis

The results are expressed as means ± SD unless otherwise indicated. The χ^2 test and Fisher's exact probability test were used to determine the statistical significance of differences between group frequencies. The corrected *P* values were obtained by multiplying the uncorrected *P* values with the number of comparisons. Group comparisons of the levels of clinical parameters were analyzed by the Mann-Whitney's *U* test or Kruskal-Wallis test in the case of more than three groups. A *P* value <0.05 was considered statistically significant.

Table 2—*Frequencies of other organ-specific autoantibodies*

	Insulin-deficient (n = 65)	Non–insulin-deficient (n = 92)	Healthy control subjects (n = 190)
TPO antibodies	32 (49.2)*†	23 (25.0)‡	22 (11.6)
TG antibodies	33 (50.8)*§	29 (31.5)‡	31 (16.3)
Parietal cell antibodies	9 (13.8)‖§	4 (4.3)	4 (2.1)
Pituitary antibodies	0 (0.0)	2 (2.2)	5 (2.6)
Any antibodies¶	44 (67.7)*#	33 (35.9)**	44 (23.2)

Data are *n* (%). *$P < 0.0001$, ‖$P < 0.001$, ‡$P < 0.005$, **$P < 0.05$ versus healthy control subjects; #$P < 0.001$; †$P < 0.005$; §$P < 0.05$ versus non–insulin-deficient; ¶number of subjects positive for one or more of organ-specific autoantibodies.

RESULTS

Clinical characteristics of adult-onset diabetic patients with GAD autoantibodies

Of the 4,980 patients with adult-onset diabetes that were screened, GADab was detected in 188 (3.8%) patients, whereas only 1 of the 190 (0.6%) control subjects was positive for GADab ($P < 0.005$). Of the 188 GADab$^+$ patients, 72 (38.3%) were classified as insulin deficient, 97 (51.6%) were non–insulin deficient, and 19 (10.1%) were unclassified because of unavailability of serum CPR data. Of these 158 GADab$^+$ patients (65 patients with insulin deficiency and 93 patients with non–insulin deficiency) were recruited for further analyses because clinical and genetic data were available (Table 1). The median level of GADab in insulin-deficient patients was 22.1 units/ml (range 1.5–39,000), which was significantly higher than that in non–insulin-deficient patients (median 5.3 units/ml, range 1.5–280,000; $P < 0.0001$).

Table 1 shows the clinical characteristics of adult-onset patients with diabetes who were positive for GADab. The age at onset of diabetes in insulin-deficient patients (41.2 ± 13.8 years) was significantly younger than that in non–insulin-deficient patients (51.3 ± 12.3 years, $P < 0.0001$). Furthermore, the frequency of insulin-deficient patients was significantly higher in patients who were <30 years old at onset of diabetes than in patients who were ≥30 years old at onset (16/19, 84.2% vs. 49/139, 35.3%, $P < 0.0001$). The frequency of family history of diabetes did not significantly differ between the two groups. The GADab$^+$ patients with insulin deficiency had a lower BMI at the time of examination, as well as lower maximum BMI, and higher HbA_{1c} level compared with non–insulin-deficient patients with GADab ($P < 0.005$). No correlation between the C-peptide levels, duration of diabetes, and anti-GADab titer was found.

Comparison of the immunogenetic characteristics between GADab$^+$ patients with insulin deficiency and non–insulin deficiency

Prevalence of IA-2ab and other organ-specific autoantibodies. IA-2ab was detected in 14 of 58 (24.1%) insulin-deficient patients and 8 of 79 (10.1%) non–insulin-deficient patients with GADab. IA-2ab was frequently detected in insulin-deficient patients ($P < 0.05$). Of the other organ-specific autoantibodies, the prevalence of anti-thyroid (TPO and TG) antibodies was significantly higher in diabetic patients with GADab, independent of their insulin secretion, compared with healthy control subjects ($P < 0.005$; Table 2). The frequency of TPO antibodies, TG antibodies, and parietal cell antibodies, but not pituitary antibodies, was increased in GADab$^+$ patients with insulin deficiency compared with non–insulin-deficient patients (Table 2). Of the anti-thyroid, parietal cell, and pituitary antibodies, 44 of 65 (67.7%) GADab$^+$ patients with insulin deficiency had one or more of these autoantibodies ($P < 0.0001$ vs. healthy con-

Table 3—*HLA-DRB1 and DQB1 allele frequencies*

Allele	Insulin-deficient (n = 130)	Non–insulin-deficient (n = 186)	Healthy control subjects (n = 380)
DRB1			
*0101	7 (5.4)	8 (4.3)	24 (6.3)
*0403	4 (3.1)*	4 (2.2)	16 (4.2)
*0405	37 (28.5)†	39 (21.0)*	43 (11.3)
*0406	1 (0.8)‡	9 (4.8)	12 (3.2)
*0802	12 (9.2)	15 (8.1)	22 (5.8)
*0803	8 (6.2)	20 (10.8)	41 (10.3)
*0901	38 (29.2)§‡	33 (17.7)	57 (15.0)
*1101	3 (2.3)	5 (2.7)	7 (1.8)
*1201	5 (3.8)	3 (1.6)	14 (3.7)
*1302	6 (4.6)	5 (2.7)	9 (2.4)
*1401	1 (0.8)	3 (1.6)	15 (3.9)
*1405	1 (0.8)	3 (1.6)	8 (2.1)
*1501	1 (0.8)*‡	9 (4.8)	36 (9.5)
*1502	1 (0.8)*‖	16 (8.6)	34 (8.9)
Other alleles	5 (3.8)	14 (7.5)	42 (11.1)
DQB1			
*0301	5 (3.8)¶	9 (4.8)¶	37 (9.7)
*0302	22 (16.9)	30 (16.1)	43 (11.3)
*0303	39 (30.0)§‡	34 (18.3)	61 (16.1)
*0401	36 (27.7)†	37 (19.9)#	43 (11.3)
*0402	1 (0.8)¶‡	11 (5.9)	19 (5.0)
*0501	7 (5.4)	8 (4.3)	28 (7.4)
*0502	0 (0.0)¶	2 (1.1)	12 (3.2)
*0503	2 (1.5)	4 (2.2)	16 (4.2)
*0601	9 (6.9)* **	38 (20.4)	75 (19.7)
*0602	1 (0.8)*	6 (3.2)¶	34 (8.9)
*0604	6 (4.6)	5 (2.7)	9 (2.4)
Other alleles	2 (1.5)	2 (1.1)	3 (0.8)

Data are n (%). †$P < 0.0001$; §$P < 0.0005$; *$P < 0.005$; #$P < 0.01$; ¶$P < 0.05$ versus healthy control subjects; **$P < 0.001$; ‖$P < 0.005$; ‡$P < 0.05$ versus non–insulin-deficient.

trols; $P < 0.001$ vs. GADab$^+$ non–insulin-deficient patients).

Frequency of HLA-DRB1 and DQB1 alleles

As shown in Table 3, the type 1 diabetes susceptibility alleles DRB1*0405 and DRB1*0901 were increased in frequency in GADab$^+$ patients with insulin deficiency compared with those in healthy control subjects. DRB1*0406, *1501, and *1502 were rare in patients with insulin deficiency. On the other hand, the frequency of DRB1*0405 but not DRB1*0901 was increased in GADab$^+$ patients with non–insulin deficiency compared with healthy control subjects. Furthermore, no difference was detected in the frequency of DRB1*1501 and *1502 alleles, which are considered to be protective from type 1 diabetes (Table 3).

DQB1*0303 and *0401 were increased in frequency in patients with insulin deficiency, whereas only DQB1*0401 was frequent in non–insulin-deficient patients compared with healthy control subjects. DQB1*0301, *0402, *0502, *0601, and *0602 were decreased in patients with insulin deficiency. In the case of GADab$^+$ patients with non–insulin deficiency, DQB1*0301 and *0602 were decreased, but no difference in the frequency of DQB1*0402, *0502, and *0601 alleles was observed, compared with healthy control subjects.

Frequency of DRB1-DQB1 haplotypes

Among the type 1 diabetes susceptible DRB1-DQB1 haplotypes reported in the Japanese population, DRB1*0405-DQB1*0401, *0802-*0302, and *0901-*0303 were increased in frequency in GADab$^+$ patients with insulin deficiency compared with healthy control subjects (Table 4). The frequency of the DRB1*0901-DQB1*0303 haplotype was also increased in GADab$^+$ insulin-deficient patients compared with GADab$^+$ non–insulin-deficient patients. However, only the DRB1*0405-DQB1*0401 haplotype was frequent in GADab$^+$ patients with non–insulin deficiency.

As expected, type 1 diabetes protective haplotypes DRB1*1502-DQB1*0601 and *1501-*0602 were rare in GADab$^+$ patients with insulin deficiency. Of note is that the frequency of the DRB1*1501-DQB1*0602 haplotype in GADab$^+$ non–insulin-deficient patients was significantly lower than in healthy control subjects, but no difference in the frequency of DRB1*1502-DQB1*0601 haplotype was observed.

To characterize the clinical and immunological features of GADab$^+$ patients with diabetes in association with HLA class II genotype, these patients were divided into five groups, based on their DRB1-DQB1 genotypes (Table 5). The patients with genotypes comprising the DRB1*0405-DQB1*0401 haplotype showed a younger age at disease onset than patients with other haplotypes. The

Table 4—*HLA-DRB1-DQB1 haplotype frequencies*

DRB1-DQB1	Insulin-deficient (n = 130)	Non–insulin-deficient (n = 186)	Healthy control subjects (n = 380)
*0405-*0401	36 (27.7)*	37 (19.9)†	43 (11.3)
*0802-*0302	11 (8.5)†	11 (5.9)	11 (2.9)
*0901-*0303	37 (28.5)‡	31 (16.7)§	56 (14.7)
*1502-*0601	1 (0.8)‡	16 (8.6)‖	34 (8.9)
*1501-*0602	1 (0.8)‡	6 (3.2)†	34 (8.9)

Data are n (%). Statistical analysis of the frequency of each HLA-DRB1-DQB1 haplotype was performed by a 2 × 2 contingency table. The corrected P values were obtained by the uncorrected P values with the number of comparisons. *$P < 0.0001$; †$P < 0.01$; ‡$P < 0.001$ versus healthy control subjects; §$P < 0.05$; ‖$P < 0.005$ versus insulin-deficient.

Table 5—*Clinical and immunological characteristics of GADab$^+$ adult-onset patients with diabetes divided by DRB1-DQB1 genotypes*

	0405-0401/0901-0303	0405-0401/X	0901-0303/Y	K/K	1501-0602/Z or 1502-0601/Z
n	11	48	44	34	21
Sex (M/F)	5/6	14/34	15/29	19/15	13/8
Age at diabetes onset (years)	44.1 ± 10.5*	45.8 ± 14.2*	46.3 ± 14.2	47.4 ± 13.1	53.4 ± 14.4
Duration of diabetes (years)	11.1 ± 8.6	9.6 ± 8.3	10.0 ± 7.5	11.8 ± 6.1	14.4 ± 12.4
BMI at examination (kg/m^2)	21.3 ± 5.0	21.2 ± 3.8*	22.5 ± 3.6	22.3 ± 3.3	23.6 ± 3.0
Insulin-deficient patients (%)	7 (63.6)†	25 (52.1)‡	22 (50.0)†	9 (26.5)	2 (9.5)
IA-2 Ab (%)	2 (18.2)	5 (10.4)	10 (22.7)	4 (11.8)	1 (4.8)
TPO Ab (%)	4 (36.4)	17 (35.4)	17 (38.6)*	14 (41.2)	3 (14.3)
TG Ab (%)	5 (45.5)	23 (47.9)	15 (34.1)	14 (41.2)	5 (23.8)
Parietal cell Ab (%)	1 (9.1)	5 (10.4)	4 (9.1)	3 (8.8)	0 (0)
GADab level (units/ml)	21.0 ± 29.1	402.2 ± 2,161.2	256.5 ± 907.3	9,659.5 ± 48,237.8	7.2 ± 8.0§

Data are means ± SE or *n* (%). X = any DRB1-DQB1 haplotype but *0901-*0303, *1501-*0602, or *1502-*0601; Y = any DRB1-DQB1 haplotype but *0405-*0401, *1501-*0602, or *1502-*0601; Z = any DRB1-DQB1 haplotype; K = any DRB1-DQB1 haplotype but *0901-*0303, *0405-*0401, *1501-*0602, or *1502-*0601. *$P < 0.05$; †$P < 0.005$; ‡$P < 0.001$ versus 1501-0602 or 1502-0601; §$P < 0.005$ versus non–1501-0602 or 1502-0601.

frequency of patients with the insulin-deficient state in patients with genotypes comprising the type 1 diabetes susceptibility haplotype was significantly higher than the other patients, even though all of these patients were GADab$^+$. A total of 21 (13.3%) of the 158 patients had genotypes comprising the type 1 diabetes protective haplotypes DRB1*1502-DQB1*0601 or DRB1*1501-DQB1*0602. These patients were characterized by high age at onset of diabetes, high BMI, resistance to insulin-deficient state, low titer of GADab, and low frequency of other organ-specific autoantibodies (Table 5).

CONCLUSIONS — This cross-sectional study was designed to investigate the clinical, autoimmune, and genetic characteristics in adult-onset (>20 years of age) diabetic patients with GADab. Autoantibodies directed to GAD constitute a major characteristic of immune-mediated form of type 1 diabetes (10,18). However, GADab can also be found in other autoimmune diseases, such as stiff-man syndrome, Graves' disease, autoimmune polyendocrinopathies, in 6–10% of patients classified with type 2 or non–insulin-dependent diabetes, as well as in 1–2% of the healthy population (13,19–22). The presence of anti-islet autoantibodies in type 2 diabetic patients suggests that they may have an autoimmune process that is responsible for their insulin-secretory deficit. In this study, the prevalence of GADab in patients with adult-onset diabetes was 3.8%, and >50% of these patients showed non–insulin-deficient stage. These results suggest that not all GADab$^+$ diabetic patients who are classified as having type 2 diabetes progress to the insulin-deficient state. Therefore, the issue of whether some factors exist that can better distinguish the nonprogressors from subjects who develop insulin deficiency needs to be clarified.

As expected, the GADab$^+$ patients with insulin deficiency showed a younger age of onset, a lower BMI at examination as well as a lower maximum BMI, and higher HbA_{1c} levels compared with non–insulin-deficient patients with GADab. Among the younger-onset patients with GADab aged <30 years of age, 84% were in the insulin-deficient state compared with 35% of patients ≥30 years of age. The prevalence of IA-2 autoantibodies and thyrogastric autoantibodies in the GADab$^+$ patients with insulin deficiency are significantly higher than those in GADab$^+$ non–insulin-deficient patients. However, anti-thyroid autoantibodies in GADab$^+$ non–insulin-deficient patients are frequent compared with healthy control subjects. These results indicate that adult GADab$^+$ patients with insulin deficiency have classical features of type 1 diabetes, similar to children, but the immunological features from GADab$^+$ patients with non–insulin deficiency are distinct. Furthermore, a younger age of onset (<30 years) might be associated with progression to insulin deficiency.

The clinical and immunological differences between GADab$^+$ patients with insulin deficiency and non–insulin deficiency were reinforced by the finding that the GADab$^+$ insulin-deficient patients also differed genetically from GADab$^+$ patients with non–insulin deficiency. In the case of GADab$^+$ patients with insulin deficiency, the frequencies of the type 1 diabetes susceptible HLA-DRB1-DQB1 haplotypes in the Japanese population (23), DRB1*0405-DQB1*0401, *0802-*0302, and *0901-*0303, were significantly higher than for GADab$^+$ patients with non–insulin deficiency and healthy control subjects. In contrast, only the DRB1*0405-DQB1*0401 haplotype was frequent in GADab$^+$ patients with non–insulin deficiency. These results are consistent with a previous report by Kobayashi et al. that concluded that DQB1*0401 was increased in patients with slowly progressive type 1 diabetes (6). Thus, DRB1*0405-DQB1*0401 may be one of the characteristic haplotypes in Japanese non–insulin-deficient patients with anti-islet autoantibodies.

Another feature that distinguishes GADab$^+$ non–insulin-deficient patients from insulin-deficient patients are the frequencies of type 1 diabetes protective HLA haplotypes. The frequency of the DRB1*1501-DQB1*0602 haplotype in GADab$^+$ non–insulin-deficient patients was significantly lower than in healthy control subjects but was higher than GADab$^+$ patients with insulin deficiency. Furthermore, no difference in the frequency of the DRB1*1502-DQB1*0601 haplotype was observed between GADab$^+$ patients with non–insulin deficiency and healthy control subjects. As shown in Table 5, 13% of the GADab$^+$ patients with diabetes had HLA genotypes composed of the DRB1*1501-DQB1*0602 or *1502-

*0601 and were characterized by high onset age, high BMI, resistance to the insulin-deficient state, low titer of GADab, and low frequency of other organ-specific autoantibodies. It has been reported that GADab$^+$ patients in the non–insulin-deficient stage have a slowly progressive form of type 1 diabetes that leads to insulin deficiency. However, our findings suggest that the GADab$^+$ non–insulin-deficient patients with DRB1*1501-DQB1*0602 or *1502-*0601 are resistant to the insulin deficiency.

It has been reported that a high titer of GADab is a good predictor of developing insulin-requiring diabetes in patients with adult-onset diabetes (22,24). In this study, however, 11 patients with a high titer of GADab (234–280,000 units/ml) remained in the non–insulin-deficient state with a mean disease duration of 8.0 ± 7.2 years (range 1.0–28.0). Furthermore, 8 of these 11 non–insulin-deficient patients with a high titer of GADab had one or more other organ-specific autoantibodies. Previous studies have demonstrated that the titer of GADab in type 1 diabetic patients with endocrine autoimmunity was extremely high and was not associated with β-cell autoimmunity (25–27). Collectively, these results indicate that the high titer of GADab may not always predict insulin deficiency in GADab$^+$ patients with adult-onset diabetes, especially for those that express other organ-specific autoantibodies.

Our observations show that GADab$^+$ non–insulin-deficient patients differ from GADab$^+$ patients with insulin deficiency with respect to clinical characteristics, humoral autoimmunity to other organ-specific autoantibodies, and HLA class II genes. Furthermore, adult-onset diabetic patients with an age of onset >30 years, HLA-DRB1*1501-DQB1*0602 or *1502-*0601, and a low-titer of GADab would be at low risk for insulin deficiency, even if they are positive for GADab.

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