Islet Cell Antibodies in Patients With Autoimmune Thyroid Disease

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Islet cell antibodies (ICAs) were assayed in 316 patients with autoimmune thyroid disease (AITD; 190 with Graves' disease, 126 with Hashimoto's thyroiditis), 53 patients with insulin-dependent diabetes mellitus (IDDM), and 144 healthy control subjects. ICAs were measured by an immunohistochemical method with peroxidase-labeled protein A and human pancreatic tissues. The prevalence of ICAs in patients with AITD was 7.6% (24 of 316), whereas the prevalence in control subjects was 0.7% (1 of 144). Among 24 ICA$^+$ patients, 20 (83%) had IDDM. In these 20 patients, the duration of diabetes from clinical onset was 5.4 ± 5.1 yr. ICAs in patients with IDDM alone were positive in 90.9% at 1 yr and 7.7% at 5 yr after the onset of diabetes. These data have shown that most ICA$^+$ patients with AITD have IDDM and that the prevalence of ICAs in patients with AITD in Japanese is as high as that found among whites, whereas the incidence of IDDM in Japanese is approximately one-thirtieth or one-fiftieth of that in whites. *Diabetes* 40:319–22, 1991

Autoimmune mechanisms play important roles in the pathogenesis of insulin-dependent diabetes mellitus (IDDM; 1). Islet cell antibodies (ICAs) were found for the first time as one of the autoantibodies to pancreatic islet cells in diabetes with autoimmune polyendocrine disease and have significant roles in the diagnosis and etiology of IDDM (2). We have reported a new method for detecting ICAs in sera with peroxidase-labeled protein A and fresh frozen human pancreatic tissues (3). ICAs were detected in 100% of Japanese IDDM patients within 6 mo after onset. The prevalence of ICAs decreased as the duration of the disease increased; however, ICAs persisted in some IDDM patients who also had autoimmune thyroid disease (AITD; 4). Organ-specific autoimmune diseases such as AITD are frequently associated with IDDM (5). On the other hand, the polyclonal production of autoantibodies such as anti-insulin autoantibodies has been reported in AITD (6). The aim of this study was to investigate the prevalence of ICAs and IDDM in a large number of AITD patients and to compare the prevalence in Japan with those of other countries where the incidence of IDDM is different from that found in Japan.

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RESEARCH DESIGN AND METHODS

During 1982–1988, 316 patients with AITD (62 men, 254 women), consisting of 190 patients with Graves' disease and 126 patients with Hashimoto's thyroiditis, and 54 patients with IDDM without AITD who were seen in the outpatient endocrinology and metabolism clinic of Nagasaki University Hospital, were screened for ICAs (Table 1). Graves' disease was diagnosed based on clinical manifestation of thyrotoxicosis with goiter and elevated serum-free thyroxine (T4) levels and thyroidal ^{123}I uptake. Hashimoto's thyroiditis was diagnosed based on $>10^4$ titers of anti-microsomal antibodies and anti-thyroglobulin antibodies and goitrous hypothyroiditis and histologically confirmed goitrous euthyroidism. The group of IDDM patients without AITD had typical juvenile-onset diabetes mellitus (11.3 ± 6.8 yr, range 2–27 yr) and did not meet the criteria for the diagnosis of AITD listed above. The diagnosis of IDDM was based on the occurrence of ketoacidosis at onset or a history of being ketosis prone, daily insulin requirements of >0.7 U · kg^{-1} · day^{-1}, daily C-peptide excretion in urine, and serum C-peptide responses to glucagon (1 mg i.v.). Table 1 shows the patients in this study. Sera from the patients were collected for 6 yr and stored at −20°C until tested. When ICA was positive, the patients were followed prospectively with a variable duration of follow-up periods.

Serum T4 and free T4 (FT4) were measured by radioimmunoassay (RIA) kits (Travenol, Tokyo). Serum triiodothy-

TABLE 1
Characteristics of patients in study

Patient	*n*	M/F	Age at onset (yr)	Age at examination (yr)
Autoimmune thyroid disease	316	62/254		45.5 ± 15.1
Graves' disease	190	50/140	37.9 ± 15.6	43.0 ± 15.1
Hashimoto's thyroiditis	126	12/114	43.6 ± 13.1	48.0 ± 15.0
Insulin-dependent diabetes without autoimmune thyroid disease	53	26/27	11.3 ± 6.8	19.1 ± 9.9
Control subjects	144	71/73		44.1 ± 18.6

Values are means ± SD.

ronine (T3), free triiodothyronine (FT3), and thyrotropin (TSH) were also measured by RIA kits (Eiken, Tokyo). The normal ranges of T4, FT4, T3, FT3, and TSH were 4.5–11.5 μg/dl, 0.6–2.3 ng/dl, 91–143 ng/dl, 2.2–6.7 pg/ml, and 0.5–5.0 μU/ml, respectively.

Anti-thyroidal microsomal antibodies and anti-thyroglobulin antibodies were measured by hemagglutination tests with commercial kits (Seroidia AMC and Seroidia ATG, Fujirebio, Tokyo) and were considered to be positive with a dilution $>1 \times 10^2$.

Urinary C-peptide excretion in 24 h and serum C-peptide responses to glucagon were measured to evaluate insulin secretion. Glucagon tests were performed after overnight fasting. Blood samples were drawn at 5 min before and at 0, 3, 5, 10, 15, and 30 min after the injection of 1 mg i.v. glucagon (Novo, Copenhagen). C-peptide was measured by RIA with commercial kits (C-peptide RIA, Daiichi, Tokyo). The assay range of C-peptide was 0.3–30 ng/ml. The criteria for insulin dependency were urinary C-peptide of <20 μg/24 h and serum C-peptide of <1.8 ng/ml at 3 min after the injection of glucagon (7–9).

ICAs were detected by an immunoenzymatic method, which was established in our laboratory (3). Staining procedures by peroxidase-labeled protein A are as follows: fresh frozen sections of human pancreatic tissues (blood type O), which had been obtained from a 53-yr-old man with gastric cancer at the time of operation, were washed three times with phosphate-buffered saline (PBS; 10 mM, pH 7.2) containing 2% bovine serum albumin (RIA grade, Sigma, St. Louis, MO). Fifty microliters of the samples was applied to the pancreatic tissues and incubated in a moist chamber for 1 h at room temperature. The tissues were washed three times in PBS. Peroxidase-labeled protein A was prepared in our laboratory: free protein A (Pharmacia, Uppsala, Sweden) was conjugated with horseradish peroxidase (Sigma), and column chromatography was performed to separate peroxidase-labeled protein A and nonbound peroxidase. Fifty microliters of peroxidase-labeled protein A (5 μg/ml) was added to the tissues and incubated for 30 min. After washing the tissues with PBS three times, they were reacted with Karnovsky's diaminobenzidine solution for 8 min at room temperature (10). The tissues were then mounted and observed under light microscopy. Positive staining was determined by cytoplasmic staining of islet cells. Anti-nuclear antibodies, anti-thyroidal autoantibodies, and anti-mitochondrial antibodies do not interfere with ICA detection when utilizing this method.

With this method, our laboratory (laboratory identification no. 135, principle investigator S.N.) participated in the ICA Proficiency Test supported by the Immunology and Diabetes Workshop in 1988 and 1989 (R.L. Dawkins, Å. Lernmark, G.S. Eisenbarth, chairmen; 11). The evaluation of our results was as follows: laboratory validity, consistency, sensitivity, and specificity were 93 and 97%, 95 and 95%, 81 and 94%, and 100 and 100%, respectively.

HLA typing for the DR antigens was performed by the standard microtoxicity test (12).

The statistical tests used were χ^2-test and Student's *t* test. The values are expressed as means ± SD. $P < 0.05$ was significant.

RESULTS

Table 2 shows the prevalence of ICAs in AITD patients and healthy control subjects. ICAs were detected in 24 of 316 patients (7.6%) and 1 of 144 control subjects (0.7%). The prevalence of ICAs in AITD patients was significantly higher than that of healthy control subjects ($P < 0.01$). When the AITD patients were divided into two groups by the presence of IDDM, ICAs were detected in 20 of 21 AITD patients with IDDM (95.2%) and 4 of 295 AITD patients without IDDM (1.4%), respectively (Table 3). The prevalence of ICAs in AITD patients with IDDM was significantly higher than that in AITD patients without IDDM (95.4 vs. 1.4%, $P < 0.01$).

Figure 1 shows the relationship between the prevalence of ICAs and duration of IDDM in the 20 ICA$^+$ AITD patients

TABLE 2
Prevalence of islet cell antibodies (ICAs) in autoimmune thyroid disease (AITD) patients and healthy control subjects

Patients	*n*	ICA$^+$ (*n*)	Positive anti-thyroidal autoantibodies (*n*)
AITD patients	316	24 (7.6)*	242 (76.6)
Graves' disease	190	14 (7.4)*	166 (87.3)
Hashimoto's thyroiditis	126	10 (7.9)*	76 (77.0)
Healthy control subjects	144	1 (0.7)	0 (0)

Values in parentheses are percentages.
*$P < 0.01$ vs. control subjects.

TABLE 3
Prevalence of islet cell antibodies (ICAs) in autoimmune thyroid disease (AITD) patients with and without insulin-dependent diabetes mellitus (IDDM)

Patients	*n*	ICA$^+$ (*n*)	Positive anti-thyroidal autoantibodies (*n*)
AITD with IDDM	21	20 (95.2)*	18 (85.7)
AITD without IDDM	295	4 (1.4)	224 (75.9)

Values in parentheses are percentages.
*$P < 0.01$ vs. AITD without IDDM.

TABLE 4
Clinical characteristics of islet cell antibody (ICA)-positive autoimmune thyroid disease (AITD) patients with insulin-dependent diabetes mellitus (IDDM)

Patient	Sex	AITD	Age at onset of IDDM (yr)	Duration of IDDM (yr)	Titers of ICA (dilution)	Titers of anti-thyroidal microsomal antibodies	HLA-D locus
1	F	Graves'	42	6	1:80	160^2	DR1
2	M	Graves'	32	8	1:80	80^2	DRw9
3	F	Graves'	16	2	1:80	40^2	NE
4	F	Hashimoto's	60	1	1:40	80^2	DRw9
5	F	Graves'	41	1	1:40	640^2	DR4
6	M	Graves'	25	3	1:40	80^2	DR4
7	M	Hashimoto's	18	9	1:40	80^2	DRw9
8	M	Graves'	40	3	1:20	−	NE
9	M	Graves'	39	3	1:20	NE	NE
10	F	Hashimoto's	34	5	1:20	40^2	DR2/DR3
11	F	Graves'	47	7	+	20^2	DR1/DR4
12	M	Graves'	37	9	+	320^2	DR4/DRw9
13	M	Hashimoto's	34	3	+	40^2	DR4
14	F	Graves'	30	1	+	40^2	DR4
15	M	Graves'	25	23	+	40^2	DRw9/DRw12
16	M	Hashimoto's	22	4	+	5120^2	NE
17	M	Hashimoto's	18	2	+	40^2	DR2
18	F	Hashimoto's	17	5	+	40^2	DR4
19	F	Hashimoto's	17	11	+	80^2	DRw8/DRw9
20	F	Graves'	13	2	+	−	NE

Mean ± SD age 30.4 ± 12.4 yr; mean ± SD duration 5.4 ± 5.1 yr. NE, not examined; −, negative; +, undiluted serum only.

with IDDM and 53 IDDM patients without AITD. In the IDDM patients without AITD, the prevalence of ICAs within 1 yr of clinical onset of the disease was 90.9% but declined with increasing duration of diabetes and was 7.7% by the 5th yr. On the other hand, the prevalence of ICAs in AITD patients with IDDM remained at 100% of those tested for at least 8 yr, although the number of AITD patients with IDDM followed for >5 yr became increasingly small compared with patients with IDDM alone.

Table 4 shows some of the characteristics of 20 ICA^+ AITD patients with IDDM. The patients consisted of 10 men and 10 women, 12 of whom had Graves' disease, and 8 had Hashimoto's thyroiditis. Their age at onset and duration of IDDM were 30.4 ± 12.4 yr (range 13–60 yr) and 5.4 ± 5.1 yr (range 1–23 yr), respectively. Half of the 20 patients had markedly elevated ICA titers that were >1:20. Seventeen patients had elevated anti-thyroidal microsomal antibody titers. There was no significant correlation between elevated titers of ICAs and anti-thyroidal microsomal antibodies. The HLA-D locus was examined in 15 patients. The prevalence of DR4 and DRw9 was 46.7% (7 of 15) and 40% (6 of 15), respectively.

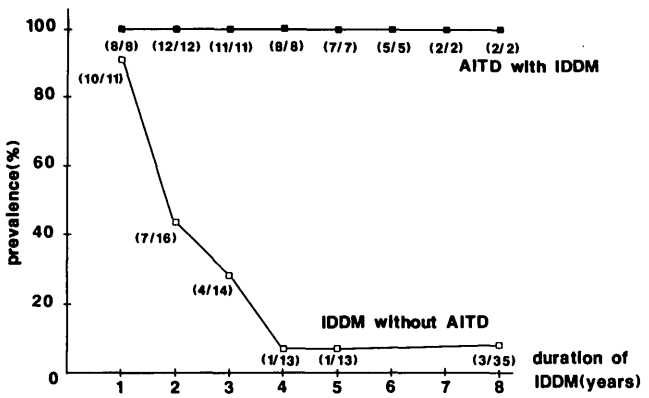

FIG. 1. Relationship between prevalence of islet cell antibodies (ICAs) and duration of insulin-dependent diabetes mellitus (IDDM). In 53 IDDM patients without autoimmune thyroid disease (AITD; □), prevalence of ICAs within 1 yr was 90.9% but declined with increasing duration of diabetes and was 7.7% by 5th yr. In 21 AITD patients with IDDM (■), prevalence of ICAs remained at 100% of those tested for at least 8 yr.

DISCUSSION

The incidence of IDDM has been reported in various parts of the world, and the lowest rate is in Japan, whereas the highest rates are in Scandinavia and the United States (13). The incidence rates per 100,000/yr of IDDM are 0.6 in Japan, 18.2 in the U.S. (whites), and 29 in Finland, respectively. ICAs were found for the first time in diabetes with autoimmune polyendocrine deficiency diseases, and ICAs were positive in 33% (10 of 30) of patients (2). Furthermore, it is known that AITD is frequently found in patients with IDDM (5). The prevalence of ICAs in a relatively small number of white insulin-treated diabetic patients with AITD was reported to be 57% (4 of 7) in thyrotoxicosis and 50% (3 of 6) in primary hypothyroidism, respectively. ICAs were detected in 2.8% (7 of 247) of AITD patients without diabetes (14). In an earlier Japanese study, the prevalence of ICAs was 50% (7 of 14) in IDDM patients with AITD (15). The number of AITD patients in previous studies has been insufficient to evaluate the actual prevalence of ICAs and IDDM in them and to compare the results with those of other countries where the incidence of IDDM is very different. In this study, ICAs were examined in 316 patients with AITD (190 with Graves' disease, 126 with Hashimoto's thyroiditis). The prevalence of ICAs in all AITD patients was 7.6% (24 of 316), and there was no difference in the patients with Graves' disease or Hashimoto's thyroiditis (7.4 vs. 7.9%). The prevalence of ICAs in AITD patients in our study in Japan was approximately the same as that observed in patients with

AITD in whites in the United Kingdom (14). The prevalence of ICAs in patients with IDDM and AITD in this study was 95% (20 of 21) and was significantly higher than that reported earlier (2,14,15). The clinical characteristics of AITD patients with IDDM in this study revealed that the age of onset of IDDM in patients with AITD were older than IDDM patients without AITD, and they had persistently elevated ICA titers. These characteristics are essentially the same as those described in previous studies in whites (14). In the analysis of the HLA-D locus of ICA^+ IDDM patients with AITD, incidence of HLA-DRw9 (6 of 15, 40%) tends to be higher than that found in Japanese control subjects (107 of 377, 28.3%; 16). Our findings are similar to a report that Japanese IDDM patients with positive anti-thyroidal antibodies have a higher frequency of HLA-DRw9 (17). Oral glucose tolerance tests were performed to examine glucose intolerance in 4 ICA^+ AITD patients. However, there was no evidence of glucose intolerance at the time of examination; a follow-up study is being performed in our laboratory.

In summary, we demonstrated that the prevalence of ICA positivity in AITD patients in Japan is as high as that found among whites, whereas the prevalence of IDDM in Japan is the lowest in the world. ICA titers remain positive in IDDM patients with AITD for a much longer duration after the onset of diabetes than in patients with IDDM alone.

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