Islet Cell Antibodies Identify Latent Type I Diabetes in Patients Aged 35–75 Years at Diagnosis

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SUMMARY

One hundred fifty-four selected patients with nonketotic diabetes diagnosed between the ages of 35 and 75 yr and treated with diet or oral hypoglycemic agents for at least 1 yr were investigated for parameters of glycemic control (weight loss, blood glucose, and glycosylated hemoglobin), islet cell function (fasting and glucagon-stimulated C-peptide responses), and immunologic markers of insulitis (total ICA and CF-ICA) or autoimmunity (thyroid and gastric antibodies). These parameters were all repeated in 9 of 22 ICA-positive patients after a 2-yr follow-up and correlated with secondary drug failure. The antibody tests were also done on 51 nondiabetic controls matched for age and body weight.

The 22 (14%) diabetic subjects having positive islet cell antibodies (ICA) included more women than men with a shorter duration of symptoms, lower body weight, more associated thyroid autoimmunity, and a tendency to have more type I diabetes in their families, although glycemic control, age at onset, and family history of type II diabetes were the same as in the 132 ICA-negative cases.

Patients with ICA had lower initial C-peptide levels and showed little rise after glucagon stimulation. Beta cell function deteriorated significantly during the 2-yr follow-up in 9 of 22 positive patients and more ICA-positive patients required insulin. It is suggested that these latent type I diabetic patients are characterized by persistent ICA, progressive loss of beta cells, and a high frequency of thyrogastric autoimmunity. The determination of ICA may be of clinical value in the diagnosis and treatment of nonketotic diabetes with onset in later life. DIABETES 1986; 35:237–41.

At onset of symptoms, most patients with type II (non-insulin-dependent) diabetes mellitus can be treated with diet and/or oral hypoglycemic agents (OHA). However, some will subsequently require insulin. For adequate control it is therefore important to identify patients who are potential secondary drug failures. Few clinical signs are associated with deterioration of beta cell function in type II diabetes. Weight loss is not obligate for insulin deficiency, and during infections or stress ketosis may be seen before insulin supplies become inadequate, so that reliable markers of future insulin dependency could be of real clinical value.

It has been suggested that the presence of islet cell antibodies (ICA) in patients with type II diabetes implies a high risk of future insulin dependency.[1,2] In addition, patients with ICA were shown to secrete less insulin in response to oral glucose than patients without them.[3] However, in these previous studies, patients were drawn from endocrine clinics and included many cases with associated thyroid disorders. Although ICA were detected by immunofluorescence (IFL), no emphasis was placed on the complement-fixing variants (CF-ICA), which are more closely related to ongoing insulitis, as they may include cytotoxic antibodies specific to beta cells. So far there are few available data of islet cell and other organ-specific antibodies in unselected type II diabetic subjects.[4,5] Prospective studies and follow-up data on the influence of ICA on beta cell function are also lacking.

The purpose of the present study was to determine the frequencies of ICA, CF-ICA, and thyrogastric antibodies in an unselected group of patients with maturity-onset diabetes and to relate them to the beta cell function of the patients. We further report a 2-yr follow-up of beta cell function and clinical course in patients with and without ICA.

SUBJECTS AND METHODS

SUBJECTS

One hundred fifty-four patients (81 females and 73 males; Table 1) with onset of nonketotic diabetes between the ages

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TABLE 1
Islet cell antibody status in relation to sex ratio, therapy, and associated autoimmune conditions in 154 maturity-onset diabetic patients

No. of patients	ICA status		Sex ratio (F/M)	Therapy			Associated autoimmune conditions in relation to onset of diabetes	
	ICA	CF-ICA		Diet (F/M)	OHA (F/M)	Ins. (F/M)		
132	–	–	67/65	6/4	47/43	14/18	F53 Thyroiditis	5 yr before
							F54 Thyroiditis	4 yr before
							F52 Thyroiditis	3 yr after
							F55 Thyroiditis	2 yr after
							F? Graves' thyrotoxicosis	1 yr before
							F66 SLE	6 yr before
11	+	–	5/6	0/0	3/3	2/3	F49 Thyroiditis	7 yr after
11	+	+	9/2	0/0	6/1	3/1	F68 Thyroiditis	2 yr after
							F? Graves' and acromegaly	3 and 15 yr before, respectively
							F73 Pernicious anemia	10 yr before
							F57 Atrophic gastritis	1 yr before
154			81/73	6/4	56/47	19/22		

Age unknown is indicated by (?).

of 35 and 75 yr were drawn from the register of the Helsinki Diabetic Association. Ten were treated with diet alone, 103 patients with diet plus oral hypoglycemic agents (OHA); 41 patients who had previously received OHA for at least 1 yr later required insulin. The clinical characteristics of the patients are shown in Tables 1 and 2. Fifty-one age- and weight-matched, nondiabetic subjects served as controls for the ICA and other autoantibody prevalence. The subjects gave informed consent for the studies and the protocol was approved by the ethical committee of Helsinki University Central Hospital.

Patients with secondary diabetes or with other diseases or drugs known to influence glucose metabolism were excluded.

METHODS

Autoantibody studies. Islet cell antibodies (ICA) were determined on all the sera by indirect immunofluorescence using cryostat sections of group 0 pancreas with undiluted patient's serum and anti-IgG or anti-C3 FITC conjugates for the detection of complement-fixing variants.[6,7] We emphasize that all ICA are now detectable with the IgG conjugates, provided that the IgG2 subclass is adequately represented.[8] The complement-fixing variants represent a proportion of the total ICA. Gastric parietal cell antibodies were similarly detected starting with undiluted sera. Thyroglobulin and thyroid microsomal antibodies were measured by hemagglutination with Wellcome Thymune T & M kits (Wellcome Laboratories, Beckenham, England).

Metabolic studies. Beta cell function was assessed by measuring C-peptide[9] before and 6 min after intravenous (i.v.) injection of 1 mg glucagon.[10] This parameter was chosen in preference to serum insulin levels, since we were interested in following possible beta cell destruction prospectively and to separate potential insulin-dependent cases from genuine type II diabetic subjects. Treatment with OHA was withheld

TABLE 2
Clinical and metabolic characteristics of patients with ICA and CF-ICA

	ICA negative	ICA positive	CF-ICA positive
N	132	22	11
Females	67	14	9*
Males	65	8	2
Family history of type II diabetes (%)	72 (55.4)	11 (52.4)	5 (45.5)
Family history of type I diabetes (%)	11 (8.5)	4 (20)	1 (10)
Age (yr)	56 ± 1	56 ± 2	58 ± 3
Age at onset (yr)	48 ± 1	52 ± 2	56 ± 3
Duration (yr)	7.4 ± 0.5	4.1 ± 0.6†	1.9 ± 0.3†
Ideal body weight (%)	124 ± 2	115 ± 4†	105 ± 5†
Fasting blood glucose (mmol/L)	9.1 ± 0.3	9.2 ± 0.5	9.8 ± 0.7
Glycohemoglobin (%)	12.2 ± 0.2	12.3 ± 0.6	12.7 ± 1.1
Basal C-peptide (mmol/L)	0.65 ± 0.04	0.35 ± 0.05†	0.32 ± 0.05†
Stimulated C-peptide (mmol/L)	1.06 ± 0.06	0.56 ± 0.09†	0.45 ± 0.07†

Data are presented as means ± SEM.
*$P < 0.05$, and †$P < 0.001$: significance of difference from ICA-negative patients.
HbA_1 glycohemoglobin normal range: 5.5–7.5% of total Hb.

TABLE 3
Frequencies of thyrogastric antibodies in relation to islet cell antibodies (ICA) in patients with maturity-onset diabetes

	ICA negative (N = 132)		ICA positive (N = 22)		CF-ICA positive (N = 11)		Normal controls* (N = 51)	
Antibody to	N	%	N	%	N	%	N	%
Thyroid microsomal (Mc)	17	12.9	10	45.5‖	5	45.5§	5	9.8
Thyroglobulin (Tg)	8	6.1	5	22.7§	3	27.3‡	5	9.8
Thyroid (Mc/Tg)	20	15.2	10	45.5‖	5	45.5‡	8	15.7
Gastric parietal cell (GPC)	29	22.0	8	36.4§	5	45.5	16	31.4†
Thyrogastric (Mc/Tg/GPC)	41	31.0	14	63.6	7	63.6‡	22	43.1

CF-ICA, complement-fixing islet cell antibodies; variants include beta cell-specific cytotoxic antibodies.
*Matched for age and weight but not for sex.
†Frequency of gastric parietal cell antibodies in normal Finnish population varies from 7% to 20% (ARO Miettinen, personal communication). Higher prevalence in the present study is possibly due to an overrepresentation of females in the control group.
‡$P < 0.05$, §$P < 0.01$, and ‖$P < 0.001$: significance of difference from ICA-negative patients.

24 h before the investigations were carried out after an overnight fast. Blood glucose was assayed by a glucose-oxidase method adapted for Autoanalyzer II (Beckman Instruments, Fullerton, California) and glycohemoglobin (HbA_1) by ion-exchange microcolumn chromatography.[11]

Follow-up studies. Ten ICA-negative (3 females and 7 males) and nine ICA-positive patients (6 females and 3 males) with similar mean age (55 ± 2 and 57 ± 3 yr, respectively) and duration of diabetes (4.1 ± 1.0 and 4.3 ± 1.1 yr, respectively) were followed during a 2-yr period for their beta cell function and persistence of ICA and CF-ICA. These 19 patients were chosen because they were willing to have the tests. All data are presented as means ± SEM. Significance of differences between means was tested with Student's *t*-tests for unpaired and paired samples where appropriate. Significance of frequency differences was tested with Fisher's exact probability test.

RESULTS

Islet cell antibodies. Positive reactions for "total" ICA were found in 22 patients (14.3%) and 11 of these sera also fixed complement when tested with anti-C3 conjugate. None of the 51 matched controls had any ICA. There was no significant difference between patients with and without ICA regarding family history of type I and type II diabetes, mean age, age at onset of diabetes, and glycemic control (i.e., fasting blood glucose and HbA_1 concentrations). However, the 22 ICA-positive cases included more females with a shorter duration of diabetes and a lower body weight. These results are detailed in Table 2. The differences between ICA-positive and -negative patients were further magnified in the subgroup of 11 cases with CF-ICA, which comprised 9 females and only 2 males. The mean duration was 1.9 yr in the 11 patients with CF-ICA, as compared with 7.4 yr in ICA-negative cases, and mean body weight was 105% versus 124% of ideal body weight. The 11 CF–ICA-positive patients also had a later onset of diabetes, 56 yr compared with 48 yr in the ICA-negative group. Most of these differences were significant to $P < 0.001$. Furthermore, in patients with complement-fixing antibodies, which contain the beta cell-specific reactions, there was a marked reduction in C-peptide concentrations (0.45 versus 1.06 nmol/L) when compared with ICA-negative patients ($P < 0.001$).

Thyrogastric antibodies. Thyroid antibodies were more frequently present in ICA-positive patients compared with ICA-negative diabetic and control subjects, 45.5% compared with 15.5% (Table 3). Gastric parietal cell antibodies were frequent in all groups tested with a maximum of 45% in the 11 CF–ICA-positive cases, in which two patients suffered from associated pernicious anemia or atrophic gastritis.

Autoimmune diseases were seen in 4 of 11 patients with CF-ICA and a further patient with ICA but no complement fixation had myxedema (Table 1). This is about three times the prevalence found in the ICA-negative group, in whom there were 6 of 132 cases with autoimmunity predominantly confined to the thyroid gland (3.8%), all in females.

Follow-up study. Two patients with thyroid antibodies developed Graves' disease and myxedema, respectively, during the 2 yr of observation. CF-ICA remained positive on follow-up. As seen in Figure 1, ICA-positive patients showed a progressive decline in their C-peptide response to glucagon and the beta cell damage was more severe in those with complement-fixing ICA. The three patients who did not fix complement had the least abnormal beta cell response and remained positive on follow-up without acquiring CF-ICA. The ICA-negative group had good starting levels of C-peptide and showed no significant decline. Six of the 22 ICA-positive patients, but only one of the 132 ICA-negative patients, were subsequently switched to insulin therapy ($P < 0.05$). The body weight of the patients did not change significantly during the follow-up period (78.4 ± 4.4 versus 78.4 ± 4.4 kg for ICA-negative patients and 68.5 ± 4.5 versus 68.8 ± 3.7 kg for ICA-positive patients, respectively, NS). The mean values for C-peptide concentrations at the beginning and at the end of the follow-up period were similar in the ICA-negative patients as in a larger, unmatched group of control individuals (N = 34, not shown).

DISCUSSION

The frequency of ICA in this group of unselected patients with maturity-onset diabetes was in the same range as reported for patients with recent diagnosis of type II diabetes and treated with OHA.[1] The frequency of CF-ICA was one-half that of the "total" ICA and none of the sera fixed complement in the absence of a positive reaction with the anti-IgG conjugate, since we now take great precautions to use conjugates having all IgG subclasses well represented in view of the unusual occurrence of ICA with restricted clonality and predominantly IgG2 antibodies. These results are consistent with previous studies in type I diabetes.[8]

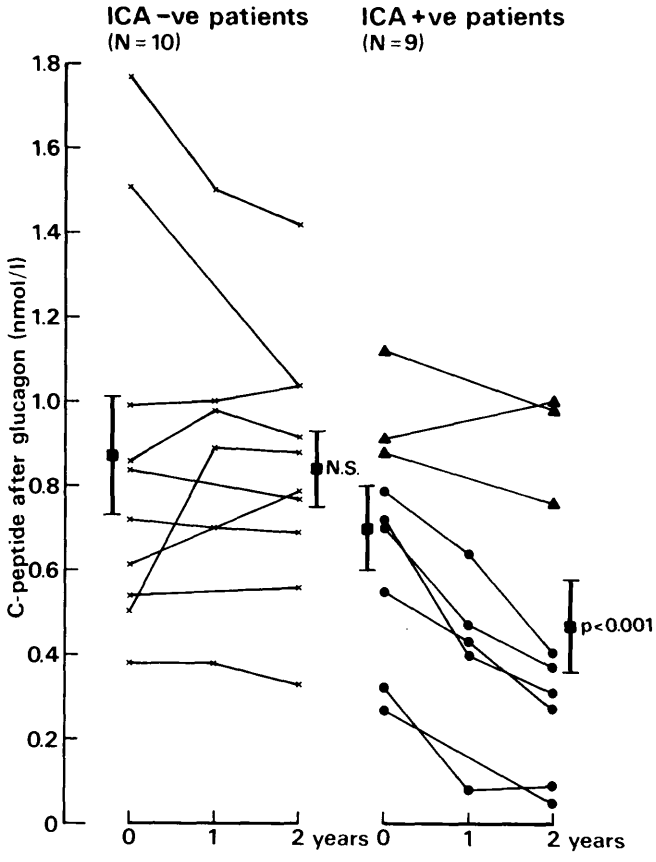

FIGURE 1. C-peptide response to 1 mg of glucagon i.v. in 10 ICA-negative and 9 ICA-positive patients with maturity-onset diabetes mellitus during a 2-yr follow-up period. The patients positive for only non-complement-fixing ICA (3 cases) are denoted by triangles and those who also showed CF-ICA (6 patients) are denoted by closed circles. The mean C-peptide values of the ICA-negative patients before and after the follow-up period were similar to those of a larger (nonmatched), ICA-negative group of patients (0.9 ± 0.05 versus 0.95 ± 0.05 nmol/L, respectively, N = 34).

The presence of ICA and CF-ICA was clearly associated with impaired beta cell function as determined by basal and glucagon-stimulated C-peptide concentrations. In contrast to insulin, C-peptide is not extracted by the liver to any significant degree and its half-life in peripheral blood is about twice that of insulin, so that it gives a more accurate measure of insulin secretory capacity.[12] Glucagon is a potent stimulator of endogenous insulin secretion,[10] and the test can be considered to measure the beta cell response during daily life.

The association of ICA to beta cell function has been the subject of two previous studies.[3,13] Insulin response to arginine was lower in ICA-positive than in ICA-negative, nondiabetic endocrine subjects.[13] No difference, however, was seen in patients with overt diabetes regarding insulin secretory capacity in relation to their ICA status.[14] In keeping with our results, Gray et al.[3] showed that ICA-positive cases had a decreased insulin response to oral glucose as compared with ICA-negative patients. The same authors presented indirect evidence for the concept that ICA is indicative of progressive beta cell deterioration by showing that ICA-positive patients more frequently developed secondary drug failure.[1,2] However, drug failure may be due to decreased insulin sensitivity as well as to impaired beta cell function.[15] The present study presents direct evidence for the view that ICA and especially CF-ICA are markers for slow, ongoing beta cell damage and, therefore, are indicative of subsequent insulin dependency.

Surprisingly, a similar long latency period was found in genetically predisposed, first-degree relatives of diabetic children unselected for autoimmunity as in the present study.[16] However, the family members who had ICA while euglycemic turned out to belong to families with increased autoimmunity. Interestingly, our ICA-positive patients reported a higher frequency of type I diabetes among their family members when compared with ICA-negative, type II diabetic subjects. Although these differences did not reach statistical significance, this positive trend needs to be explored further.

Some predisposed relatives lost their CF-ICA with time and its disappearance correlated with a normal glucose metabolism for up to 6 yr.[17] However, persistence of ICA (? high titer[18]) in pre-type I diabetic patients correlated with a linear loss of beta cell function.[19] It can be envisaged that, in our present series, the islet cell damage was more severe and advanced as reflected by the patients being already diabetic and by the persistence of the antibodies.

ICA tests further identify a subgroup of maturity-onset diabetic subjects who are predominantly female, who tend to be of normal weight or are slightly underweight, and are found to have a high frequency of other organ-specific antibodies and autoimmune diseases. This group again reinforces the concept of heterogeneity in diabetes.[20] Since their clinical course differs from that of most type II diabetic subjects, they must be considered as potentially suffering from type I diabetes despite the late onset and slow progression of the disease.

Therefore, the introduction of a new subgroup of latent type I diabetic subjects "hidden" in the type II diabetic population may be warranted.[21] These "maturity-onset insulin-dependent" diabetic subjects are characterized by the presence of ICA, an excess of HLA-DR3 or -DR4,[4] and a high frequency of thyrogastric antibodies with progressive beta cell deterioration. In the present series, we also have preliminary results of an excess of DR3 and DR4 in the non-insulin-dependent, ICA-positive patients as compared with our ICA-negative group. The determination of ICA may be of clinical value in diagnosis and treatment of patients with onset of diabetes mellitus in later life.

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