Clinical and subclinical organ-specific autoimmune manifestations in Type 1 (insulin-dependent) diabetic patients and their first-degree relatives

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Summary. Studying 239 Type 1 (insulin-dependent) diabetic patients and 144 of their first-degree relatives, we found a significant prevalence of autoimmune manifestations in both groups, compared with sex- and age-matched control subjects ($p < 0.001$). In particular, in diabetic patients we found a high frequency of autoimmune thyroid disease and idiopathic Addison's disease and also a significant prevalence of thyroid ($p < 0.001$), parietal cell ($p < 0.05$) and adrenal antibodies ($p < 0.05$). In the relatives a high frequency of thyroid disease, thyroid, parietal cell and adrenal antibodies and a significant prevalence of islet cell antibodies ($p < 0.05$) were detected. In

both groups functional glandular tests and gastric biopsies performed on the basis of autoantibody positivity revealed 13 examples of subclinical hypothyroidism, two cases of reduced adrenocortical reserve and five of atrophic gastritis. Autoantibody screening in diabetic patients and their relatives permitted the early diagnosis of the underlying endocrine disorders.

Key words: Organ-specific autoimmunity, indirect immunofluorescence, islet cell antibodies, Type 1 diabetes, autoimmune diabetes.

Type 1 diabetes is commonly associated with thyroid autoimmune disease, autoimmune atrophic gastritis, pernicious anaemia and idiopathic Addison's disease [1–6]; Schmidt's syndrome also can be a complication of Type 1 diabetes [7]. Furthermore, Type 1 diabetic patients without clinical manifestations of organ-specific autoimmune disease have a markedly increased incidence of thyroid microsomal, gastric parietal cell, intrinsic factor and adrenal autoantibodies in their sera [1, 8–14]. This increased incidence of autoantibodies was particularly pronounced in young females [9] and in diabetic patients over the age of 35 years [15]. It has been demonstrated also that the persistence of islet cell antibodies (ICA) for more than 3 years in Type 1 diabetic patients is associated with a high prevalence of thyroid microsomal, parietal cell and adrenal antibodies [15–17]. Some authors, however, have not found an increased incidence of organ-specific autoantibodies in diabetic patients [18, 19].

Studying first-degree relatives of Type 1 diabetic patients, a significant prevalence of thyrogastic [10] and ICA [16, 20, 21] has been found. The relatives of diabetic probands with autoimmunity had a significantly higher frequency of autoimmune manifestations than relatives of probands without autoimmunity [10, 16, 22].

The aim of this study was to evaluate the prevalence of clinical and subclinical autoimmune manifestations in a group of Type 1 diabetic patients and their relatives and to study possible correlations between probands and families with and without autoimmunity.

Subjects and methods

Diabetic patients

We studied 130 male and 109 female Type 1 diabetic patients, aged 2–67 years (mean 19 years). The time since diagnosis ranged from 1 week to 12 years (mean 28 weeks). Eighty of these patients were followed-up at 3 monthly intervals for a mean period of 3.5 years from the onset of diabetes.

Relatives

We studied 61 male and 83 female first-degree relatives of Type 1 diabetic patients, aged 10–63 years (mean 35 years). The number of parents was 107, siblings 35 and offspring two. In particular, 94 of the 144 subjects represented 37 entire families. In every family two or more non-diabetic relatives were studied.

Normal subjects

We examined two groups of control subjects, matched for sex, age and race: (1) 135 males and 115 females, aged 2–71 years (mean

20 years); (2) 155 males and 206 females, aged 8–60 years (mean 37 years). In this way we have compared for autoantibody prevalence a population of diabetic patients with a similar population of normal controls, and a population of first-degree relatives with a similar population of controls. The control subjects were selected from healthy individuals in the population at large: healthy blood donors, medical students, hospital employees and paediatric patients attending clinics for minor trauma.

All subjects were examined and anyone with a personal or familial history for autoimmune disease was excluded from the control groups. All serum samples were tested for the presence of organ-specific autoantibodies.

Immunological study

We determined thyroid microsomal, gastric parietal cell and adrenal antibodies by the indirect immunofluorescence technique on normal human unfixed tissues [23]. ICA-IgG were detected by the method of Bottazzo et al. [24] on cryostat sections of normal human pancreas of blood group 0+, using a fluorescein conjugated sheep anti-human IgG antiserum (Wellcome Reagents, Beckenham, Kent, UK) at 1:40 dilution. Complement-fixing-ICA (CF-ICA) were detected on the same substrate, according to Bottazzo et al. [25], using a normal human serum as source of complement and a fluorescein conjugated rabbit anti-human-C3 antiserum (Behringwerke, Marburg, FRG) at 1:40 dilution. IgG antibodies reacting against glucagon- and somatostatin-producing cells in the islets were searched for by the indirect immunofluorescence technique proposed by Bottazzo et al. [26] and the corresponding complement-fixing antibodies were detected using an indirect immunofluorescent complement fixation test. Thyroglobulin autoantibodies at $\geq 1:80$ dilution were determined by a tanned red cell agglutination using a commercial kit (Wellcome).

Functional tests

The following tests were performed in subjects having organ-specific autoantibodies without clinical evidence of the relevant autoimmune disease: (a) in patients with thyroid microsomal and/or thyroglobulin antibodies, thyroid function was assessed by determination of thyroid hormone plasma levels and by the TRH-test (basal TSH levels [normal values: 0–4 $\mu\text{U}/\text{ml}$] and TSH levels 20 min [10–30 $\mu\text{U}/\text{ml}$] and 60 min [5–20 $\mu\text{U}/\text{ml}$] after intravenous injection of 200 μg of synthetic TRH); (b) in patients with adrenal antibodies, adrenocortical function was assessed by determination of ACTH plasma levels (normal values: <22 pmol/l), basal cortisol levels (normal values: 138–690 nmol/l) and cortisol levels after intravenous injection of 0.25 mg of tetracosactide esacetate (Ciba-Geigy, Basel, Switzerland); (normal increase >100% of basal values at 60 min); (c) in non-diabetic subjects with ICA, glucose tolerance was evaluated by an oral glucose tolerance test; (d) the subjects with glucagon-cell antibodies were studied by an arginine-test (100 mg of L-arginine intravenous over 30 min; determination of blood glucose and glucagonaemia at times 0, 5, 15, 30, 60, 90 min); (e) the patients positive for gastric parietal cell antibodies were studied by gastric biopsy. Prior consent was obtained from all subjects. TSH, T_3 , T_4 and cortisol plasma levels were measured by a specific radioimmunoassay using kits supplied by Diagnostic Products (Los Angeles, California, USA), ACTH plasma levels using a kit by Cea-Sorin (Vercelli, Italy) and glucagon plasma levels by a radioimmunoassay method using K 30 antiserum supplied by Dr. R. Unger (Dallas, Texas, USA).

Statistical study

Statistical significance was assessed using the χ^2 test with Yates' correction for continuity if the number in any expected class was five or less. Fisher's exact test was used if any class was 0.

Table 1. Overt and latent organ-specific autoimmunity in Type 1 diabetic patients and in their first-degree relatives

	Autoimmune cases						Autoimmune manifestations					
	Polyendo-			Thyroid			Gastric			Adrenal		
	No.	%	crine ^a only	Over ^b	Antibodies only ^c	Total	Over ^d	Antibodies only ^c	Total	Over ^e	Antibodies only ^c	Total
Diabetic patients	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total (n=239)	50 ^f	21	10	4	3	37 ^f	16	1	13 ^g	5	4	10 ^f
Males (n=130)	23 ^f	18	3	2	1	15 ^f	11	0	7 ^g	5	1	4
Females (n=109)	27 ^h	25	7	6	5	17	16	1	6	3	3	6 ^h
Control subjects	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total (n=250)	16	6	1	—	—	11	4	—	4	2	—	0
Males (n=135)	3	2	0	—	—	1	1	—	0	—	—	0
Females (n=115)	13	11	1	1	—	10	9	—	4	4	—	0
Relatives	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total (n=144)	31 ^f	22	4	3	2	16	11	—	6	4	—	2
Males (n=61)	5	8	0	—	—	2	3	—	1	2	—	0
Females (n=83)	26 ^h	31	4	5	3	14	17	—	5	6	—	2
Control subjects	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Total (n=361)	35	10	2	1	0	25	7	—	7	2	—	1
Males (n=155)	5	3	0	—	—	2	1	—	0	—	—	0
Females (n=206)	30	15	2	1	0	23	11	—	7	3	—	1

^a Cases with more than one autoimmune manifestation (either overt or with antibodies only); ^b Graves' disease, Hashimoto's thyroiditis, idiopathic hypothyroidism; ^c absence of overt disease; ^d pernicious anaemia; ^e idiopathic Addison's disease; ^f $p < 0.001$; ^g $p < 0.05$; ^h $p < 0.01$

Results

Diabetic patients

Organ-specific autoimmunity. Fifty diabetic patients (21%) had one or more clinical or latent autoimmune manifestations (Table 1). This frequency was highly significant compared with the frequency in the sex- and age-matched control subjects ($p < 0.001$). Statistical significance was found both in males ($p < 0.001$) and females ($p < 0.01$). In 10 cases (4%), more than one autoimmune manifestation was present (autoimmune polyendocrinopathy).

Islet cell antibodies. The prevalence of ICA appeared to be related to the duration of Type 1 diabetes. Out of 222 diabetic patients over 6 years of age, 116 (52%) were ICA-IgG-positive and 77 (35%) also had CF-ICA. After 3 years duration of disease, 66 patients (30%) remained ICA-IgG-positive and 26 (12%) CF-ICA-positive. Out of the 17 diabetic children under 6 years of age, who were tested within 4 weeks of diagnosis of diabetes, 10 (59%) were ICA-IgG-positive and six (35%) also had CF-ICA. After 3 years, two children (12%) were still ICA-positive. No diabetic patient had antibodies reacting against individual glucagon- or somatostatin-producing cells only.

Thyroid autoimmunity. Clinical or latent autoimmune manifestations against the thyroid were found in 37 patients (16%; $p < 0.001$). This result was significant both for males ($p < 0.001$) and females ($p < 0.01$). Seven patients presented with clinical disease: four had Graves' disease, two Hashimoto's thyroiditis and one idiopathic hypothyroidism. The remaining 30 patients (13%; $p < 0.001$) had only thyroid microsomal and/or thyroglobulin antibodies in the absence of clinical thyroid disease. This frequency was significant only in the males ($p < 0.001$). Out of these 30 patients, seven had elevated basal TSH levels (mean 15.9 $\mu\text{U/ml}$) and elevated levels at 20 and 60 min after TRH injection (mean 51.9 and 44 $\mu\text{U/ml}$, respectively); two patients had normal basal TSH levels but a hyper-response to TSH after TRH injection (mean 44.7 $\mu\text{U/ml}$ at 20 min and 40.8 $\mu\text{U/ml}$ at 60 min). In all these nine patients T_3 and T_4 levels were normal; substitutive therapy with l-thyroxine was started prophylactically.

Gastric autoimmunity. Thirteen patients (5%) had gastric autoimmune manifestations ($p < 0.05$): one had pernicious anaemia and 12 had parietal cell antibodies in the absence of anaemia ($p < 0.05$). Statistical significance was found only in the males ($p < 0.05$). Only one patient was studied by gastric biopsy and he had focal atrophic gastritis.

Adrenal autoimmunity. We found autoimmune manifestations against the adrenal cortex in 10 subjects (4%;

Fig. 1. Significant incidence of organ-specific autoimmunity in ICA-positive diabetic patients (■) with duration of the disease > 3.5 years with respect to the ICA-negative diabetic patients (▨), and in the latter group compared with control subjects (□)

$p < 0.001$): four had overt idiopathic Addison's disease; the other six (3%; $p < 0.05$) had adrenal antibodies without clinical evidence of adrenal insufficiency. At the beginning of the study, two of these six patients had normal ACTH levels (mean 8.9 pmol/l) and normal basal cortisol levels (mean 354 nmol/l), but a low cortisol response to an ACTH-test (mean 496 nmol/l). In one of these patients, impaired adrenocortical reserve progressed to overt Addison's disease after 25 months of observation.

Follow-up studies on patients. Of 80 diabetic patients, 28 remained persistently ICA-positive after a mean period of 3.5 years. Sixteen of these persistently ICA-positive subjects (57%) had one or more autoimmune manifestations, but the latter were present in only eight of 52 diabetic patients (15%) with negative or transiently positive-ICA. The difference in prevalence between the two groups was statistically significant ($p < 0.001$). A significantly higher frequency of subjects with autoimmune manifestations was found also in the group of ICA-negative diabetic patients compared with control subjects ($p < 0.05$; Fig. 1).

Relatives

Organ-specific autoimmunity. Thirty-one relatives (22%) had one or more clinical or latent autoimmune manifestations ($p < 0.001$). Compared with the sex- and age-matched control subjects, this prevalence was statistically significant only in females ($p < 0.01$). In four cases (3%) an autoimmune polyendocrinopathy was found.

Fig. 2. Correlations between familial and individual organ-specific autoimmunity in diabetic patients. The incidence of autoimmunity in the diabetic probands was significantly higher in families with at least one non-diabetic member with autoimmune manifestations (■) compared with families without such manifestations (▨) ($P < 0.01$).

Fig. 3. Correlations between organ-specific autoimmunity in the diabetic probands and in their families. The incidence of autoimmune families was significantly higher in the group of diabetic patients with autoimmunity (■) than in diabetic patients without autoimmune manifestations (▨) ($P < 0.001$).

Thyroid autoimmunity. Clinical or latent autoimmune manifestations against the thyroid were found in 19 subjects (13%; $p < 0.05$). Statistical significance was found only for females ($p < 0.05$). Three patients had Graves' disease; in the other 16 (11%) thyroid autoantibodies only were detected. Four of these 16 patients (25%) had high basal TSH levels (mean $18.2 \mu\text{U/ml}$) and high levels at 20 and 60 min after TRH injection (mean 77 and $69.8 \mu\text{U/ml}$, respectively). In all four cases T_3 and T_4 were normal; however, substitutive therapy with l-thyroxine was started prophylactically.

Gastric autoimmunity. Six non-anaemic subjects (4%) were positive for parietal cell antibodies. In four of these (67%) a gastric biopsy revealed histological signs of atrophic gastritis; in two normal mucosa was found.

Adrenal autoimmunity. Adrenal antibodies were detected in two subjects (1%) with normal ACTH levels, normal cortisol basal levels and normal cortisol response to an ACTH-test at the onset and also after 3 years of adrenal antibody persistence.

Pancreatic autoimmunity and diabetes. Islet cell autoimmunity was present in eight relatives (6%; $p < 0.01$); five had ICA-IgG (4%; $p < 0.05$), of which two had also CF-ICA, and three had glucagon cell antibodies (2%), two of which were complement fixing antibodies. Statistical significance was found for females only ($p < 0.01$). In all these cases the oral glucose tolerance test and glucagon increments during arginine infusion were normal. Five of the 37 whole families studied (14%) had a second or third diabetic member in addition to the proband.

Correlation between familial and individual autoimmunity in diabetic probands. Out of 37 whole families studied, 15 (41%) had at least one non-diabetic member with organ-specific autoimmunity; the remaining 22 (60%) lacked autoimmune manifestations. Nine (60%) diabetic probands of these 15 autoimmune families also had autoimmune manifestations; however, autoimmunity was present in only three (14%) probands of 22 families without such manifestations. The difference between these two frequencies was statistically significant ($p < 0.01$). In the 37 families studied 12 (32%) diabetic probands with autoimmunity were found (Fig. 2).

Correlations between autoimmunity in the diabetic proband and in the family. Considering the 37 diabetic probands of the whole families studied, 12 had autoimmune manifestations and nine of these (75%) had at least one autoimmune relative. An autoimmune relative was also present in seven (28%) of the 25 remaining families in which the diabetic patients were without autoimmunity. The difference between these two frequencies was statistically significant ($p < 0.001$). For the total 37 diabetic probands an autoimmune relative was found in 16 cases (43%; Fig. 3).

Discussion

Our data confirm that in Type 1 diabetes the prevalence of ICA decreases with the duration of the disease. In diabetes of more than 3 years duration, ICA was found in about one-third of the patients. Statistically there was no significant difference in the prevalence at onset of

ICA in diabetic patients under or over the age of 6 years. This result confirms the recent observation of Barbosa et al. [21] and disagrees with previous studies [16, 17]. However, after 3 years duration of disease, the difference of ICA-prevalence between the above subgroups was nearly threefold. In diabetic patients we did not find antibodies reacting with single cell types in the islet, probably because the presence of ICA could have a masking effect. In the families of Type 1 diabetic patients the predisposition to diabetes is revealed by the high incidence (14%) of more than one case of Type 1 diabetes. On the other hand, the tendency to pancreatic autoimmunity in the relatives is confirmed by the significant prevalence of ICA and the high frequency of glucagon cell antibodies. At the beginning of our family studies, normal glucose tolerance and normal glucagon plasma levels were found in the relatives with ICA and glucagon cell antibodies, respectively. It will be important to follow-up ICA-positive patients, especially if they are CF-ICA, because it is well established that these antibodies are good markers of β -cell damage and permit identification of subjects at risk for diabetes mellitus [20, 25, 27, 28]. It will also be interesting to follow up the subjects with glucagon cell antibodies, even if it has not yet been demonstrated that the presence and persistence of these autoantibodies may reveal a state of glucagon deficiency [29, 30].

In our study we found a high prevalence of thyroid autoimmune disease in both groups and an elevated frequency of Addison's disease in diabetic patients. The observed frequencies are particularly significant when considering the low mean age of the subjects studied. In diabetic patients without clinical autoimmune disease significant prevalence of thyroid, gastric parietal cell and adrenal antibodies were found. In the relatives without clinical autoimmune disease we found high frequencies (two to five times that in the control group) of thyroid, parietal cell and adrenal antibodies and a significant prevalence of ICA. Considering the sexes separately, our study revealed a significantly increased prevalence of thyrogastric antibodies in diabetic males and of ICA in female relatives. In many cases the autoantibodies were detected at the onset of diabetes, but in some cases they appeared in subsequent samples. On the basis of positivity for autoantibodies in the absence of any other clinical sign of illness, functional glandular tests or gastric biopsies showed that cases of subclinical hypothyroidism, latent Addison's disease and latent atrophic gastritis existed in both groups. In one diabetic patient subclinical adrenocortical insufficiency progressed to overt Addison's disease during the study period. These data agree with the prospective study of Riley et al. [11, 31] regarding the presence of biochemical hypothyroidism in diabetic patients with thyroid antibodies and with the prospective study of Betterle et al. [32] regarding the onset of adrenal insufficiency in subjects with adrenal antibodies. Riley et al. [33] have elucidated the value of gastric parietal cell

antibodies for predicting patients at risk for atrophic gastritis and cobalamin deficiency. Type 1 diabetes tends to be complicated by organ-specific autoimmune diseases particularly if ICA persist for more than 3 years (Fig. 1). The early detection of latent organ-specific autoimmune deficiencies is particularly important in diabetic patients because such manifestations, if not treated promptly, slowly progress to overt glandular insufficiency and can affect the course of the illness and its complications (e.g. hypothyroidism induces subnormal growth in young diabetic patients and hypercholesterolaemia increases vascular damage; hypocortisolism modifies insulin requirement; anaemia aggravates the already compromised state of the diabetic patient).

Another point which emerges from our study is that a high frequency of autoimmunity (28%) exists in the families of diabetic patients who do not have autoimmune manifestations themselves (Fig. 3). Nevertheless, a greater percentage of autoimmunity (75%) was present in families with autoimmune diabetic probands. These statistics suggest that it is important to screen family members of cases with Type 1 diabetes for autoimmune manifestations (even when these are not present in the probands). In many cases early diagnosis of underlying disorders may be obtained and treated, or subjects at increased risk of developing overt autoimmune diseases may be followed periodically with subsequent reduction in morbidity. Although the financial implications of autoantibody screenings are great, at present the socialized medicine system in Italy is well equipped to provide such screening on a mass scale at low cost.

In conclusion, diabetes mellitus can be accompanied by clinical or latent autoimmune manifestations to a significant degree in the proband and his family members. Our data support the hypothesis of Bottazzo et al. [16] that some forms of diabetes mellitus may be primary autoimmune entities, the characteristics of which are the persistence of ICA and the presence of individual and familial autoimmune manifestations.

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