Anti-pancreatic autoimmunity and Graves' disease: study of a cohort of 600 Caucasian patients

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Abstract

The aim of this study was to investigate the frequencies of clinical diabetes and humoral markers of anti-pancreatic autoimmunity in a homogeneous population of 600 Caucasian patients with recently diagnosed Graves’ disease (GD), in order to characterize the specific features of this group of endocrine patients among subjects at risk of diabetes.

Ten were already diabetic at GD diagnosis. Among the 590 non-diabetic patients, 29 had islet cell antibodies (ICA), including 15 with low titre ICA and only 1 ICA-positive subject with a familial history of diabetes. Twenty-four patients had insulin autoantibodies, including three in association with ICA. Glutamic acid decarboxylase (GAD)/64 kDa antibodies were found in 16 of the 150 tested sera, including 13 of the 29 ICA-positive sera. Four ICA-positive patients displayed 37/40 kDa antibodies, including three in association with GAD/64 kDa antibodies. During follow-up, one of the ICA-positive patients developed insulin-dependent diabetes, 14 years after the GD diagnosis.

To summarize, this anti-pancreatic autoimmunity study was focused on a large but specific and homogeneous group of subjects at risk for diabetes: recently diagnosed GD patients. This population was characterized by a high prevalence of GAD/64 kDa antibodies but also by a low frequency of evolution towards diabetes and the slowness of the process which could be due to the fact that only a minority of subjects possessed a sufficient combination of anti-pancreatic markers at the same time.

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Introduction

Subjects at risk of developing type 1 diabetes mellitus may be identified by the detection of anti-pancreatic humoral markers (1, 2). An increased risk of type 1 diabetes mellitus was reported in patients with other endocrine autoimmune diseases, including thyroid diseases (3). This risk was initially studied by determining islet cell antibodies (ICA) and insulin autoantibodies (IAA) (3–5). The proportion of polyendocrine ICA-positive patients who developed type 1 diabetes was dramatically smaller than ICA-positive first degree relatives, another high risk population (6). Antibodies to glutamic acid decarboxylase (GAD)/64 kDa protein have also been detected in diabetic and non-diabetic endocrine patients (7–10). More recently, the predictive value of 37/40 kDa antibodies, identified as IA2 and phogrin proteins (11–15), has been reported in non-diabetic patients with organ-specific autoimmune disease (16). Very few of these studies previously published on anti-pancreatic immunity in Caucasian endocrine patients, were homogeneous with regard to the initial disease and the intensity of extra-pancreatic autoimmunity at the time of sampling and studied the whole range of humoral markers available. In our clinic of endocrine diseases, we have a large recruitment of patients at diagnosis of Graves’ disease (GD) (17–20) and most anti-pancreatic markers (ICA, IAA, GAD/64 kDa, 37/40 kDa autoantibodies) are available in our laboratory. The aim of this study was to investigate the frequencies of clinical diabetes and humoral markers of anti-pancreatic autoimmunity in a homogeneous population of 600 Caucasian patients with recently diagnosed GD in order to characterize the specific features of this group of endocrine patients among subjects at risk of diabetes.

Subjects, materials and methods

Six hundred serum samples from patients (85% women, 15% men; age: 41 ± 23 years) with newly diagnosed GD were retrospectively tested for anti-pancreatic humoral markers. The patients were consecutively recruited in the Department of Endocrinology of the Rennes Teaching Hospital, between 1980 and 1994. They were enrolled in studies on GD management, after giving their informed consent (17–20) according to protocols approved by the
local Ethics Committee. All were Caucasian and living in Western France. GD diagnosis was based upon the usual clinical and biological signs including determination of free thyroid hormones and thyroid stimulating hormone, as well as scintigraphic criteria (homogeneous thyroid scans). All patients received carbimazole for 6–36 months according to various protocols (17–20). The sera were collected upon diagnosis, before initiation of any specific treatment. Serum aliquots were stored at −20°C, handled in the same manner, and the various samples were thawed only once. After examination of the patients’ medical records, two groups were formed according to the presence or absence of diabetes (WHO criteria).

Antibody screening was performed as follows:
(1) In all samples, ICA and IAA were detected.
(2) Antibodies to glutamic acid decarboxylase (GAD), to 64 kDa protein and to 37/40 kDa protein were determined in the sera of diabetic patients at GD diagnosis (n = 10): ICA and/or IAA-positive, non-diabetic patients (n = 50); a group of ICA and/or IAA-negative, non-diabetic patients (sex and age-matched to the ICA and/or IAA-positive group, n = 100).
(3) ICA heterogeneity was analysed from sera with an ICA titre >20 Juvenile Diabetes Foundation (JDF) units. (4) In the non-diabetic ICA and/or GAD/64 kDa positive group (n = 32), a follow-up of ICA and GAD/64 kDa antibodies was performed on sera collected in the course of the GD management protocols. For these patients, clinical data were also obtained from a questionnaire filled out by general practitioners or endocrinologists. Recent fasting glycaemia was required in all cases for diabetic diagnosis.

ICA determination on human pancreas
ICA were detected by indirect immunofluorescence on sections of a human frozen pancreas as previously described (21). Sera were also tested after prolonged (18h) incubation in the presence of aprotinin (22). Results were expressed in JDF units. One ICA-positive internal standard and the international reference sera from the International Workshops were included (23).

Our laboratory contributed to the periodical proficiency tests from 1989 to 1996 and in the last Workshop obtained 100% sensitivity and 100% specificity with a detection threshold of 2.5 JDF units.

Insulin autoantibodies
IAA were measured by RIA (24), using the ability to bind monoiodinated 125I-TyrA-14 human insulin (specific activity 250 μCi/μg, Novo, Copenhagen, Denmark). The inter- and intra-assay variation coefficients were <15%. A positive internal standard sample was included in each assay. A positive result was defined as a value >1.2%, i.e. >3 S.D. above the mean binding of control sera (0.90±0.10%). These control sera exhibited normally distributed values. In the last IAA Workshop, our laboratory obtained 72% sensitivity and 100% specificity.

Humoral reactivity to GAD
Two methods were used to detect humoral reactivity to GAD.

Antibodies to the 64 kDa islet antigen (Figure 1)
Antibodies to the 64 kDa islet antigen were determined using a variant of the immunoprecipitation method described by Christie et al. (25). Briefly, islets were isolated from pancreases of 7-day-old Wistar rats by collagenase digestion. Extracts labelled with [35S]methionine (Amersham, Amersham, Bucks, UK) were precipitated with the serum to be tested and the immune complexes were bound to protein A–Sepharose (Pharmacia, Uppsala, Sweden).

After separation by SDS-PAGE, all fluorographs were independently analysed by two observers unaware of the sample identity. Negative and positive control sera (kindly provided by M R Christie) were included in each experiment.

Antibodies immunotrapping brain GAD activity
Antibodies to GAD were also measured by determining the enzyme activity immunotrapped by sera from a soluble extract of adult rat brain as previously described (7, 26, 27). Briefly, brain extracts from adult Wistar rats were incubated with the serum to be tested. Immune complexes isolated on protein A–Sepharose were submitted to [1-14C]glutamic acid and 14CO2 release was then measured by scintillation counting. GAD activity was computed as the percentage of the activity trapped by the same standard antibody-positive control serum used in analyses of 64 kDa antibodies. Sera were considered positive when the activity was greater than 6%, i.e. greater than 3 S.D. of the activity in sera from 82 control subjects (mean±S.D. 3±1%). The inter-assay and intra-assay coefficients of variation were 11% and 8% respectively. In the First (Lab. identification n°18) and Second International GAD Antibody Workshops (Lab. identification n°49), our laboratory had respectively 100% and 66% sensitivity and 100% and 97% specificity at a blind analysis of serum samples (28, 29). As previously described (30), the method was also validated by comparison with a radioligand assay using in vitro translated human [35S]methionine-labelled GAD 65. In a series of 54 sera, 50 were concordant in the two assays. Two sera were positive by immunotrapping and negative in the radioligand assay and for the two others the discrepancy was opposite.

Antibodies to 37/40 kDa (Figure 1)
Radiolabelled insulinoma cells were prepared as described for 64 kDa antibody determination. The pellet was processed for trypsinization at 0.2 mg/ml.
trypsin (Type XIII, Sigma Immunochemicals, L’Isle d’Abeau, France) as previously described (26, 31). Trypsin extracts were precleared with normal human serum before and after binding by protein A–Sepharose. Aliquots of extract were incubated with serum of patients or controls (negative and positive, kindly provided by M R Christie). As a control of sensitivity, a 1/16 dilution of a positive serum, which was established as the limit of detection of our assay, was included in each experiment. After separation by SDS-PAGE, all fluorographs were independently analysed by two observers without knowledge of sample identity for 37/40 kDa. None of 50 control tested sera was positive.

Study of islet cell antibody heterogeneity
Experimental blocking of ICA reactivity by recombinant GAD
The blocking effect of GAD on ICA reactivity in human pancreas was analysed on sera with ICA >20 JDF units (32), using recombinant GAD 65 (rGAD) obtained in baculovirus and purification by affinity chromatography on nickel columns (kindly provided by P Van Erdert, INSERM U25, Paris). To determine the dose of recombinant GAD necessary for the blocking experiments, GAD was incubated at various doses with patients’ sera containing ICA >80 units and a high level of GAD antibodies. Ten microlitres serum were incubated with 10 μg rGAD. Serum and an equal amount of added PBS were incubated overnight at 4°C as internal control. ICA was titrated by immunofluorescence on human pancreas. A positive blocking effect was defined as a loss of fluorescence activity equal to or greater than 2-fold dilutions.

Four-layer immunofluorescence technique (33)
Sections of human pancreas were first incubated for 20 min with patient’s undiluted sera, washed in PBS and incubated for 20 min with fluorescein isothiocyanate labelled goat anti-human IgG (Biosys, Compiègne, France) diluted to 1/20 in PBS. Then the sections were incubated for 20 min with monoclonal antibody directed against either human glucagon (GLU-001, Novo-Nordisk, Copenhagen, Denmark) diluted to 1/2, or undiluted human pro-insulin (HPI-005, Novo-Nordisk). The fourth layer consisted of an incubation with biotinylated horse anti-mouse Ig (Vector Laboratories Inc., Burlingame, CA, USA) diluted to 1/40. The anti-hormone antibodies were visualized by incubating the sections with tetramethyl-rhodamine isothiocyanate labelled avidin D (Vector Laboratories) diluted to 1/80 in PBS. Two distinct patterns were individualized: a whole pattern where α and β cells were equally marked, and a β cell predominant pattern.

Statistical analysis
The statistical significance of antibody level differences was determined using the Mann–Whitney non-parametric test. The significance of frequency differences was determined by chi-square analysis with Yates’ correction or by Fisher’s exact test.

Results
Diabetic population among recently diagnosed GD patients
In this large cohort of 600 patients, 10 were diabetic at the time of GD diagnosis (1.7%; 7 women, 3 men). In three cases, GD and diabetes were diagnosed simultaneously. Four patients had a familial history of type I diabetes mellitus. Nine were insulin-dependent, eight since diabetes diagnosis and one 1 year after. The clinical and immunological characteristics of these patients are summarized in Table 1.

Immunological markers in the non-diabetic population
ICA, IAA frequencies Twenty-nine non-diabetic patients with newly diagnosed GD (Table 2) were

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Table 1 Clinical and immunological data of diabetic patients at the onset of GD.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age at onset of diabetes (years)</th>
<th>Insulin dependency at onset of GD</th>
<th>Age at onset of GD (years)</th>
<th>Duration of diabetes at onset of GD (years)</th>
<th>ICA (JDF units)</th>
<th>IAA</th>
<th>GAD antibody (% positivity)</th>
<th>64 kDa antibody</th>
<th>37/40 kDa antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>34</td>
<td>ID</td>
<td>34</td>
<td>0</td>
<td>40</td>
<td>–</td>
<td>– (+52%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>F</td>
<td>58</td>
<td>ID</td>
<td>58</td>
<td>0</td>
<td>40</td>
<td>+</td>
<td>+ (+10%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>F</td>
<td>56</td>
<td>NID</td>
<td>63</td>
<td>7</td>
<td>0</td>
<td>–</td>
<td>– (3%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M</td>
<td>43</td>
<td>ID</td>
<td>54</td>
<td>11</td>
<td>0</td>
<td>–</td>
<td>+ (7%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M</td>
<td>28</td>
<td>ID</td>
<td>68</td>
<td>40</td>
<td>0</td>
<td>+</td>
<td>– (2%)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>F</td>
<td>23</td>
<td>ID</td>
<td>23</td>
<td>0</td>
<td>5</td>
<td>+</td>
<td>+ (7%)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>F</td>
<td>28</td>
<td>ID</td>
<td>37</td>
<td>9</td>
<td>0</td>
<td>+</td>
<td>+ (8%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M</td>
<td>33</td>
<td>ID</td>
<td>35</td>
<td>2</td>
<td>0</td>
<td>+</td>
<td>+ (8%)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>F</td>
<td>47</td>
<td>ID</td>
<td>48</td>
<td>1</td>
<td>40</td>
<td>–</td>
<td>+ (86%)</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

a ID, insulin dependency; NID, non-insulin dependency.
found to be ICA-positive (4.9%). Fifty sera were weakly positive (≤5 JDF units) and 12 exhibited a positivity ≥20 JDF units. These patients were 22 women and 7 men, aged 39±15 years (14–68 years). The age and sex ratios of the 29 ICA-positive subjects were the same as in our overall population of 590 patients. Only one ICA-positive patient had a familial history of type 1 diabetes mellitus. Twenty-four of these 590 non-diabetic subjects were IAA-positive (4%), three of whom were also ICA-positive.

**GAD/64 kDa antibodies** Among the whole population tested (n=150), 16 were positive for GAD/64 kDa antibodies with the following distribution: 13/29 ICA-positive subjects, 1/21 IAA-positive/ICA negative subject and 2/100 ICA-negative/IAA-negative patients. Ten of the 13 ICA-positive and GAD/64 kDa-positive patients were recruited among the 12 subjects with ICA ≥20 units. Three of the 24 IAA-positive subjects had GAD/64 kDa antibodies but two of them had also ICA.

**Heterogeneity of ICA** All eight sera with ICA >20 units were totally or partially blocked by preincubation with rGAD. The eight sera were GAD-positive. No whole pattern was found. All had a β cell predominant pattern.

**Antibodies to 37/40 kDa** Among the 150 tested sera, antibodies to 37/40 kDa were found in 4 ICA-positive sera, 1 of which was also IAA positive. In three cases, they were associated with ICA ≥20 JDF units and GAD/64 kDa antibodies. None of the ICA-negative/IAA negative patients who were age and sex matched to ICA and/or IAA positive patients was 37/40 kDa-positive.

**Follow-up in the ICA and/or GAD/64 kDa-positive non-diabetic group** During a clinical follow-up period ranging from 1 to 14 years (median 5 years), all 32 ICA and/or GAD/64 kDa-positive non-diabetic patients were always euglycaemic, except one who developed acute insulin-dependent diabetes 14 years after the first analysis.

A follow-up of humoral markers (ICA, GAD/64 kDa antibodies) was performed on available sera collected during the GD management protocol. In patients with ICA <20 units, no change was observed in the positivity and/or level of ICA. All subjects with ICA ≥20 units were persistently positive. Furthermore, ICA levels in that group were stable (variation ±1 dilution) except in three cases: in one patient, the level fell from 320 to 40 units in 4 years. In the other two cases the titre dropped two dilutions in 6 months and 2 years, respectively.

### Table 2 Clinical and immunological characteristics of ICA-positive GD patients.

<table>
<thead>
<tr>
<th>ICA (units)</th>
<th>Sex</th>
<th>Age (years)</th>
<th>ICA (JDF units)</th>
<th>IAA</th>
<th>GAD (% positivity)</th>
<th>64 kDa antibody</th>
<th>37/40 kDa antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤5</td>
<td>F</td>
<td>37</td>
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<td>–</td>
<td>– (3)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>24</td>
<td>5</td>
<td>–</td>
<td>– (3)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>47</td>
<td>5</td>
<td>–</td>
<td>– (2)</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>68</td>
<td>5</td>
<td>–</td>
<td>– (2)</td>
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<td>–</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>37</td>
<td>5</td>
<td>–</td>
<td>– (4)</td>
<td>–</td>
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<td>52</td>
<td>5</td>
<td>–</td>
<td>– (5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>61</td>
<td>5</td>
<td>–</td>
<td>– (3)</td>
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</tr>
<tr>
<td></td>
<td>F</td>
<td>46</td>
<td>5</td>
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<td>– (4)</td>
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</tr>
<tr>
<td></td>
<td>M</td>
<td>31</td>
<td>5</td>
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<td>– (4)</td>
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<td>F</td>
<td>41</td>
<td>5</td>
<td>+</td>
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</tr>
<tr>
<td></td>
<td>M</td>
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<td>– (3)</td>
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<tr>
<td></td>
<td>M</td>
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<td>– (2)</td>
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</tr>
<tr>
<td>10–20</td>
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<tr>
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<td>– (5)</td>
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<td>20</td>
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<td>– (3)</td>
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<td>80</td>
<td>+</td>
<td>+ (65)</td>
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</tr>
<tr>
<td></td>
<td>F</td>
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<td>320</td>
<td>–</td>
<td>+ (17)</td>
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</tr>
<tr>
<td></td>
<td>F</td>
<td>66</td>
<td>320</td>
<td>+</td>
<td>+ (66)</td>
<td>+</td>
<td>+</td>
</tr>
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</table>
sera, except one, with GAD/64 kDa antibodies were persistently positive. This sera at the limit of the positivity (6%) for GAD antibodies became negative in 1 year. The three GAD/64 kDa-positive but ICA-negative sera remained unchanged.

In the case of the woman who became diabetic, all markers except IAA were positive at GD diagnosis, 1 year after and at the onset of diabetes, without major changes in levels (Table 3). Upon diagnosis of diabetes, a \(\beta\) cell predominant pattern was always seen and ICA fluorescence was partially blocked by preincubation of the serum with recombinant GAD.

**Discussion**

An increased risk of type 1 diabetes mellitus was reported in autoimmune endocrine patients (3). Clinical diabetes was found in 1.7% of our 600 Caucasian recently diagnosed GD patients. This prevalence was higher than in the French background population (<0.3%), which confirmed the association between these two autoimmune diseases. Few data are available about the frequency of diabetes mellitus in patients with a particular type of thyroid autoimmune disease such as GD. Mouradian & Abourizk in a 1983 literature review (34) reported a 2.3–3% incidence of diabetes in all types of subjects with hyperthyroidism.

In the non-diabetic population, the prevalence of ICA (4.9%) was also significantly higher than in the general population of our country (<1%), or even than in French schoolchildren (1.8%) (35) but was not very different from the prevalence of ICA in relatives of diabetic patients (36). This prevalence of ICA was slightly higher than reported in other studies on Caucasian and Japanese patients (37–39). This increased positivity percentage could be explained by use of a more sensitive assay and also perhaps by the time chosen for screening: the subjects were all sampled at the time hyperthyroidism was diagnosed, when autoimmunity phenomena were not only active, but perhaps enhanced (40).

It is now well established that the 64 kDa protein identified by immunoprecipitation method is GAD (7) and we have previously demonstrated that antibodies to the 64 kDa protein and to GAD were strongly associated in diabetic patients and their ICA-positive relatives (36, 41). The Second GAD Workshop showed that the mean sensitivity of radiobinding assays (76.2%) was higher than for ELISA (36.5%) and immunotrapping assays (49.9%) (29). Even if the sensitivity of our anti-GAD immunotrapping assay was higher than the mean sensitivity of this method (66%), we chose to increase

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**Table 3** Evolution of immunological markers for patient who progressed to IDDM.

<table>
<thead>
<tr>
<th>At onset of GD</th>
<th>Years after GD diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (IDDM onset)</td>
</tr>
<tr>
<td>ICA (JDF units)</td>
<td>40</td>
</tr>
<tr>
<td>IAA</td>
<td>–</td>
</tr>
<tr>
<td>GAD antibody (% positivity)</td>
<td>+(76)</td>
</tr>
<tr>
<td>64 kDa antibody</td>
<td>+</td>
</tr>
<tr>
<td>37 kDa antibody</td>
<td>+</td>
</tr>
</tbody>
</table>

ND, not determined; IDDM, insulin-dependent diabetes mellitus.

**Figure 1** Autoradiographs showing immunoprecipitation of the 37/40 kDa protein from \([^{35}S]\)methionine-labelled insulinoma cells (a) and 64 kDa protein from \([^{35}S]\)methionine-labelled islets (b) with sera from newly diagnosed GD patients (lanes G), positive control serum (lane C+) and negative control sera (lane C–).

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the efficiency of the detection of the reactivity to GAD by combining two tests: immunotrapping assay and immunoprecipitation of 64 kDa protein. GAD/64 kDa antibodies were found in 16 of the 150 GD patients tested, often associated with high ICA titres. In newly diagnosed GD patients prevalence of anti-GAD reactivity was increased compared with our control population (1.2%) and other groups of subjects at risk of developing diabetes, schoolchildren (<1%) (42) and first-degree relatives of diabetic patients (about 4%) (43).

Increased prevalence of GAD antibodies in autoimmune thyroid populations has also been shown in previous publications (9, 10). In these reports very few patients were positive for ICA and the potential association between these two markers could not be investigated. In our study, the prevalence of GAD/64 kDa antibodies in the ICA-positive group reached 45%. In addition, in subjects with high titres of ICA, GAD itself is also recognized by ICA, as shown by the predominant β cell pattern of the ICA-positive sera and the partial or total inhibition of ICA reactivity after preabsorption with recombinant GAD. These data are in accordance with previous works showing a strong reactivity to GAD in patients whose diabetes was associated with a thyroid autoimmune disease (8).

The origin of this reactivity should be discussed. It has been shown that GAD is not exclusively present in the brain and pancreas, and that it can be found in many other tissues, including the thyroid gland. Autoimmune reaction against thyroid GAD cannot be entirely ruled out, but GAD is only present in minute amounts (44), and is perhaps also immunologically different from the brain and pancreatic GAD, as observed for the GAD found in other organs like liver, kidney, adrenal and pituitary glands (44). However, the analysis of the pathogenic potential of GAD antibodies and their relationship to the development of clinical diabetes requires long-term studies.

No association was found between the IAA on the one hand and either the ICA or GAD antibodies on the other hand. In endocrine autoimmune patients, the presence of IAA could correspond to autoimmune epiphenomena (45). However, the IAA positivity, especially when associated with ICA positivity, could also suggest an implication of insulin as an autoantigen in the autoimmune aggression. The epitopes recognized in these two situations may differ (46). Additional studies involving a large number of IAA-positive patients would allow a test of that hypothesis.

Antibodies to 37/40 kDa which recognize tyrosine phosphatase proteins IA2 and phogrin (11–15) were only detected in 4 of the 150 tested patients. These 4 positive patients also displayed ICA and 3 of them GAD/64 kDa antibodies. One of them developed insulin-dependent diabetes many years later. Although 37/40 kDa antibodies have been reported as markers of rapid evolution towards clinical diabetes in this endocrine autoimmune population (16), 37/40 kDa antibodies were already present 14 years before diabetes was diagnosed. The strongly associated anti-GAD reactivity could perhaps explain this slow evolution to clinical expression of the disease (30).

The follow-up study confirmed that the proportion of endocrine patients with ICA who developed type 1 diabetes was substantially lower than observed in ICA-positive first-degree relatives: in our experience, only 1 of the 29 ICA-positive subjects, including those carrying several markers in high titres, developed clinical diabetes. Several facts can account for such a situation: first of all, it is very likely that the presence of anti-pancreatic autoimmunity does not necessarily induce an evolution towards clinical diabetes (47), as illustrated in this study by the prevalence of ICA being higher than the number of expected diabetes. It is also a known fact that clinical diabetes may develop very slowly in this type of patient (6). In those included in our study, the duration of follow-up varied between subjects, depending when they were taken under our management, and the only metabolic marker used was fasting blood glucose. It cannot be ruled out that some of these subjects may slowly develop diabetes with time, and that they may already carry infraclinical metabolic disorders such as a low first-phase insulin release, which was not investigated.

Although ICA alone have a moderate predictive value, this marker could nonetheless help to screen patients with a higher risk of developing diabetes mellitus among the endocrine population (4, 5, 10, 39). In these ICA-positive subjects, other factors have been reported to promote evolution towards diabetes, including 37/40 kDa positivity (6, 16) but also whole pattern ICA (6, 39). A relationship has been found between these two factors: ICA are a complex of several antibodies directed against different antigens including GAD (32) and 37/40 kDa protein (13). In opposition to whole pattern ICA which recognize several specificities and have a high predictive value, β cell predominant pattern preferentially recognizes GAD antigen and has a moderate predictive value. This latter pattern has been found in the majority of ICA-positive endocrine patients who do not progress to diabetes (6, 39, 47) and in all our patients. So, a large autoimmune reactivity could characterize the ICA-positive endocrine patients who progressed towards diabetes. Familial history of diabetes could also be of major importance (5) but was not found in our ICA-positive patients except one. Furthermore, among ICA-positive endocrine patients, specific genetic features could influence evolution towards clinical diabetes (48).

In conclusion, this anti-pancreatic autoimmunity study was focused on a specific and homogeneous but large group of subjects at risk of diabetes, recently diagnosed GD patients. This population was characterized by a high prevalence of GAD/64 kDa antibodies, but also by a low frequency of slow evolution towards diabetes and the slowness of the process which
could be due to the fact that only a minority of subjects had a sufficient combination of anti-pancreatic markers at the same moment.

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