Factors associated with the presence of glutamic acid decarboxylase and islet antigen-2 autoantibodies in patients with long-standing type 1 diabetes

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Abstract

Aim. – Glutamic acid decarboxylase (GAD) and/or islet antigen-2 (IA-2) autoantibodies (ab) are present in 90% of patients at the onset of type 1 diabetes (T1D). Few studies have shown that they may persist in the long-term. We analysed the frequency of GADab and IA-2ab and the factors associated with their persistency in patients with long-lasting T1D.

Methods. – This cross-sectional study included 430 adult patients with T1D of at least 10-year duration, consecutively seen over one year. GADab and IA-2ab were determined by radio-binding assays. Autoantibodies to thyroperoxidase, gastric parietal cells and transglutaminase were assessed in 418 patients, and HLA DRB1 genotyping in 359. Parameters associated with the persistency of antibodies were studied by multivariate analysis.

Results. – Median age at diagnosis of T1D was 12 years, and median diabetes duration was 19 years. Extrapancreatic autoimmunity was present in 38% of the patients, and associated autoimmune diseases in 21%. GADab and/or IA-2ab were found in 56% of the patients, and in 32% in those with more than 25-year diabetes duration. GADab were more frequent than IA-2ab. Female sex, an older age at diagnosis, and a shorter duration of diabetes were independently associated with the presence of ab. The same factors and the DR3 allele were associated with GADab, while only diabetes duration and the DR4 allele were associated with IA-2ab.

Conclusion. – In a large proportion of the patients with T1D, the long-term persistency of diabetes-associated antibodies allows aetiological diagnosis, even far from the onset of hyperglycaemia.

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Keywords: Type 1 diabetes; GAD antibodies; IA-2 antibodies; Persistency; Classification

Résumé

Facteurs associés à la persistance à long terme des autoanticorps dirigés contre la décarboxylase de l’acide glutamique et l’antigène d’ilot de type 2 au cours du diabète de type 1.

Objectif. – Les autoanticorps (Ac) ant décarboxylase de l’acide glutamique (GAD) et/ou antigène d’ilot de type 2 (IA-2) sont présents chez 90% des patients à la découverte d’un diabète de type 1 (DT1). Quelques études ont montré qu’ils peuvent persister à long terme. Nous avons étudié la fréquence des GADAc et des IA-2Ac et les facteurs associés à leur persistance chez des patients ayant un DT1 évoluant depuis au moins dix ans.

Méthodes. – Cette étude transversale a concerné 430 patients. GADAc et IA-2Ac ont été recherchés par radioéclat. Les marqueurs d’auto-immunité extrapancréatique étaient disponibles chez 418 patients et un génotype des allèles de DRB1 chez 359. Les facteurs associés à la persistance d’Ac ont été identifiés par analyse multivariée.

Résultats. – L’âge médian au diagnostic était de 12 ans et l’ancienneté du diabète de 19 ans. Des marqueurs d’auto-immunité extrapancréatique étaient présents chez 38% des patients et une maladie auto-immune associée chez 21%. Un Ac au moins a été trouvé chez 56% des patients et chez 32% de ceux ayant un diabète de plus de 25 ans d’ancienneté. Les GADAc étaient plus fréquents que les IA-2Ac. Le sexe féminin, un âge plus élevé au diagnostic et un diabète plus récent étaient associés à la présence d’Ac. Ceux facteurs et l’allèle DR3 étaient associés aux GADAc, alors que seuls l’ancienneté du diabète et l’allèle DR4 étaient associés aux IA-2Ac.

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1. Introduction

In type 1 diabetes mellitus (T1D), major targets of autoantibodies (ab) and T-lymphocyte include glutamic acid decarboxylase 65 (GAD), Islet Antigen-2 (IA-2), insulin and zinc transporter-8 (Zn-T8) [1]. Antibodies directed to these antigens are sensitive and specific markers of T1D [2]. At clinical onset of T1D, the combined prevalence of GADab and/or IA-2ab is 90 to 95% [3].

Other diabetes subtypes may clinically mimic T1D. Identification of diabetes subtype may entail practical consequences, even long after onset of the disease [4]. In this context, the detection of anti-islet cell antibodies might be of value for aetiological diagnosis [2]. Previous studies have shown that, although the prevalence and titers of GADab and IA-2ab decline with time along the course of T1D, some patients may retain diabetes-associated ab in the long-term [5–11].

We have analyzed the factors associated with the persistency of GADab and IA-2ab in patients with T1D with duration ranging from 10 to 65 years.

2. Patients and methods

2.1. Patients

This retrospective cross-sectional study included 430 consecutive patients with T1D of at least 10-year duration seen over one year in our department. The diagnosis of T1D was established on the association of: an age less than 20 years, and/or the presence of ketosis, and/or the presence of autoantibodies at the onset of diabetes, and strict insulin dependency from the onset. The study was approved by the Institutional Review Board Comité de Protection des Personnes Ile-de-France III.

2.2. Methods

GADab and IA-2ab were determined by commercial radio-binding assays, according to manufacturer’s recommendations (Cisbio Bioassays, 30200 Bagnols sur Cèze, France). In the 2005 Diabetes Antibody Standardization Program, the sensitivities of the GADab and the IA-2ab assays were 84% and 70%, and the specificities were 95% and 100%, respectively [12].

Ab to thyperoxydase were assayed by ELISA (The Binding Site Ltd, Birmingham, England), ab to gastric parietal cells by indirect immunofluorescence, and ab to transglutaminase (IgG and IgA) by ELISA (Biorad, Marnes-la-Coquette, France). Extrapancreatic autoimmunity was defined as the presence of at least one of these ab, with or without overt disease. Associated autoimmune diseases were defined by clinical and/or biological, and/or histological anomalies, together with the corresponding ab.

Among the 430 patients, 73% were of EuroCaucasian origin. No difference was found in the distribution of the various analysed HLA DR genotypes in Eurocaucasian and non-Eurocaucasian patients. Thus, the two groups were combined for statistical analyses.

For each patient, the mean of the last three HbA1c values (HPLC, normal 4.3–5.7%) measured over the last year was calculated. Insulin requirements (UI/kg/d) were calculated as the mean of daily doses during the week preceding each HbA1c measurement, and the three values were averaged.

2.3. Statistical analysis

Results were expressed as absolute numbers and percentages, or median and range. Comparisons between groups were performed by chi² analysis and non-parametric tests where appropriate. P-values less than 0.05 were considered significant. Factors associated with the presence of ab were assessed by multivariate logistic regression models, with separate models for the presence of either GADab or IA-2ab, and for each antibody specificity.

3. Results

3.1. Main characteristics of the patients

The characteristics of the 430 patients are shown in Table 1. Median diabetes duration was 19 years. In 21% of the patients, one or several associated autoimmune diseases were present, mainly thyroid diseases (75% of the cases), and a further 17% had at least one extrapancreatic autoantibody. Autoimmune diseases and extrapancreatic autoimmunity were more frequent in women than in men (29% vs. 12%, and 49% vs. 24%, respectively, P<0.0001).

3.2. Prevalence of anti-GAD and anti-IA-2 antibodies

At least one antibody was found in 56% of the patients, with one antibody in 42% and the two antibodies in 14% (Table 1). GADab were more frequent than IA-2ab (196 patients, 46%, vs. 106 patients, 25%, P<0.0001) (Table 2). Among the patients with a single detected antibody, GADab were also more prevalent than IA-2ab (135 vs. 45 patients, P<0.0001).

3.3. Factors associated with the presence of antibodies

In the univariate analysis, GADab and/or IA-2ab were more frequent in women than in men (63% vs. 49%, P=0.0035)
(Table 1). This was due to a higher prevalence of GADab in women (56% vs. 34%, \(P<0.0001\)), while that of IA-2ab was similar in women and in men (23% vs. 27%) (Table 2). Patients with antibodies were older at diagnosis and had a shorter duration of the disease than those with no antibody (Table 1). According to diabetes duration, a time-dependent decline in the prevalence of antibodies was observed: 73% in patients with 10–14 year duration, 60% in those with 15–19 years, 55% in those with 20–24 years, and 32% in those with more than 25 years (\(P=0.0001\)), with similar trends for GADab and IA2ab (Fig. 1). However, in patients with diabetes-associated antibodies we observe no significant decline of GADab or of IA-2ab titers according to diabetes duration (data not shown). Antibodies were more frequent in patients with extrapancræatic autoimmunity (64%) than in those with no associated autoimmunity (52%, \(P=0.033\)). We found no difference in the prevalence of GADab or IA-2ab according to the presence of clinical autoimmune diseases compared to that of extrapancræatic ab

<table>
<thead>
<tr>
<th>Sex: F/M</th>
<th>430</th>
<th>189</th>
<th>180</th>
<th>61</th>
<th>241</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA haplotypes</td>
<td>359</td>
<td>162</td>
<td>149</td>
<td>48</td>
<td>197</td>
</tr>
<tr>
<td>DR 3/4</td>
<td>114 (32%)</td>
<td>50 (31%)</td>
<td>48 (32%)</td>
<td>16 (33%)</td>
<td>64 (32%)</td>
</tr>
<tr>
<td>DR 3/non 4</td>
<td>79 (22%)</td>
<td>29 (18%)</td>
<td>42 (28%)</td>
<td>8 (17%)</td>
<td>50 (25%)</td>
</tr>
<tr>
<td>DR4/non 3</td>
<td>136 (38%)</td>
<td>67 (41%)</td>
<td>47 (32%)</td>
<td>22 (46%)</td>
<td>69 (35%)</td>
</tr>
<tr>
<td>DR non3/non4</td>
<td>30 (8%)</td>
<td>16 (10%)</td>
<td>12 (8%)</td>
<td>2 (4%)</td>
<td>14 (7%)</td>
</tr>
<tr>
<td>EPA: +/-</td>
<td>158/260</td>
<td>58/124</td>
<td>75/101</td>
<td>25/35</td>
<td>100/136</td>
</tr>
<tr>
<td>AAD: +/-</td>
<td>87/331</td>
<td>29/153</td>
<td>47/129</td>
<td>11/49</td>
<td>58/178</td>
</tr>
</tbody>
</table>

Data are actual numbers with percentage into parentheses or median with range into brackets.

ab: antibody; EPA: extrapancræatic autoimmunity; AAD: associated autoimmune disease.

Table 2
Factors associated with the presence of diabetes-associated antibody specificities in 430 patients with type 1 diabetes.

<table>
<thead>
<tr>
<th>GADab + 196</th>
<th>GADab– 234</th>
<th>(P) for GAD+ vs. GAD–</th>
<th>IA-2ab+ 106</th>
<th>IA-2ab– 324</th>
<th>(P) for IA-2+ vs. IA-2–</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex F/M</td>
<td>125/71</td>
<td>99/135</td>
<td>&lt;0.0001(a)</td>
<td>51/55</td>
<td>173/151</td>
</tr>
<tr>
<td>Age at diagnosis (yrs)</td>
<td>13.5 [1–70]</td>
<td>10 [1–39]</td>
<td>&lt;0.0001</td>
<td>12.5 [2–59]</td>
<td>11 [1–70]</td>
</tr>
<tr>
<td>Age at test (yrs)</td>
<td>33.5 [18–83]</td>
<td>32 [18–81]</td>
<td>0.15</td>
<td>31 [18–70]</td>
<td>33 [18–83]</td>
</tr>
<tr>
<td>HLA haplotypes</td>
<td>158</td>
<td>201</td>
<td>87</td>
<td>272</td>
<td></td>
</tr>
<tr>
<td>DR 3/4</td>
<td>53 (34%)</td>
<td>61 (30%)</td>
<td>27 (31%)</td>
<td>87 (32%)</td>
<td></td>
</tr>
<tr>
<td>DR 3/non 4</td>
<td>45 (28%)</td>
<td>34 (17%)</td>
<td>13 (15%)</td>
<td>66 (24%)</td>
<td></td>
</tr>
<tr>
<td>DR4/non 3</td>
<td>50 (32%)</td>
<td>86 (43%)</td>
<td>41 (47%)</td>
<td>95 (35%)</td>
<td></td>
</tr>
<tr>
<td>DR non 3/non4</td>
<td>10 (6%)</td>
<td>20 (10%)</td>
<td>0.019</td>
<td>6 (7%)</td>
<td>24 (9%)</td>
</tr>
<tr>
<td>EPA: +/-</td>
<td>88/104</td>
<td>70/156</td>
<td>0.0017(b)</td>
<td>37/67</td>
<td>121/193</td>
</tr>
<tr>
<td>AAD: +/-</td>
<td>52/140</td>
<td>35/191</td>
<td>0.0037(c)</td>
<td>17/87</td>
<td>70/244</td>
</tr>
</tbody>
</table>

Data are actual numbers with percentage into parentheses or median with range into brackets.

ab: antibody; EPA: extrapancræatic autoimmunity; AAD: associated autoimmune disease.

\(a\) OR 2.42, 95% CI 1.64–3.58.

\(b\) OR 1.91, 95% CI 1.28–2.88.

\(c\) OR 2.07, 95% CI 1.28–3.34.
without overt disease (data not shown). The distribution of HLA DR genotypes was similar in patients with and without antibodies (Table 1).

Median HbA1c (7.8 vs. 8.0%) and insulin requirements (0.73 vs. 0.72 UI/kg/d) were not different in patients with or without antibodies. In the subgroup of patients with acceptable metabolic control, defined by a mean HbA1c value less or equal to 7.5% in the absence of severe hypoglycaemia, we found no difference in daily insulin needs of the patients with at least one ab (0.74 ± 0.21 UI/kg/d) and those with no ab (0.71 ± 0.18 UI/kg/d, \( P = 0.46 \)).

In the multivariate analysis (Table 3), female sex, an older age at diagnosis, and a shorter duration of diabetes were associated with the presence of at least one antibody, while extrapancreatic autoimmunity and HLA DR genotypes were not. Female sex, an older age at diagnosis, a shorter duration of diabetes, extrapancreatic autoimmunity and the presence of the HLA DRB1*04 allele were all significantly associated with GADab. Only a shorter diabetes duration and the presence of the HLA DRB1*04 allele were associated with IA-2ab (not shown).

4. Discussion

Our study shows that GADab and/or IA-2ab are frequently detected in patients with long-standing T1D. Previous studies have shown that, in patients tested 4 to 10 years after onset of the disease, the prevalence of islet-cell antibodies, GADab or IA-2ab ranged 65–85% [5,7,11]. However, few studies have reported the prevalence of antibodies in the very long-term. In 38 patients with a mean disease duration of 20 years, the prevalence of GAD and/or IA-2ab was 66% [6]. In 282 patients, 31% tested positive for these antibodies after a mean of 26-year diabetes duration [9]. In the Joslin Medalist Study, 30% of 374 patients were antibody positive after a mean 56-year duration of diabetes [10]. Our results are in keeping with these studies since at least one antibody was found in 56% of the patients after a 19-year median duration of the disease, and even in 32% after 25 years.

Our study has several flaws. Due to its retrospective design, antibodies were not available at diagnosis in many subjects. However, the results observed in the subset of patients with antibody positivity at diagnosis were similar to that of the entire cohort (not shown). Also, we did not assess Zn-T8ab. However, a recent study showed that Zn-T8ab declined rapidly after onset of the disease and that they were detected in only 1.4% of long-standing T1D patients [9]. Lastly, we only assessed the three extrapancreatic autoimmune markers reported as the most frequently present in patients with T1DM. Thus, rare associated autoimmune diseases may have been missed by this analysis. However, all overt autoimmune diseases were systematically recorded in our patients, and among the entire cohort, only nine patients had an autoimmune disease not associated with the three markers.

As in some but not all other studies, the long-term prevalence of GADab was higher than that of IA-2ab. However, in our study this was already the case at the first time point analysis, and no difference was found in the rate of decline of the two antibodies (Fig. 1). This may be due to a lower sensitivity of the IA-2ab assay, to a lower prevalence of IA-2ab at onset, or to a faster decline of IA-2ab within the first 10 years of the disease, compared to GADab. Several factors were associated with the long-term presence of autoantibodies. As expected, duration of the disease was negatively associated with the persistency of both GADab and IA-2ab, the DR3 allele was associated with the presence of GADab, and the DR4 allele with that of IA-2ab [11]. An older age at diagnosis was independently associated with persistent GADab, as previously reported [6,7,11]. Female sex was associated with the presence of diabetes antibodies, mainly because of its association with GADab. This may reflect a general tendency to stronger autoimmunity in women than in men. Furthermore, in women but not in men, the presence of the DR3 allele was associated with an increased prevalence of both GADab and extrapancreatic autoimmunity, defining an autoimmunity-prone subgroup among patients with T1D (Table 4).
The significance of antibody persistency long after the onset of T1D remains unclear. It has been hypothesized that the long-term persistency of B-cells may sustain a chronic autoimmune response [5,6]. Indeed, endogeneous insulin secretion was detected in a large proportion of T1D patients, even decades after onset of the disease [10,13], and the presence of B-cells has been evidenced in patients dead after 42–84 years of diabetes [10]. However, no correlation was found between the presence of B-cells and that of antibodies [10]. Similarly, analyses of the relationship between residual insulin secretion and persistent antibodies led to conflicting results [8–10,13]. In our study, insulin secretion was not assessed, but daily insulin requirements and HbA1c levels were similar in patients with and without persistent antibodies. Alternatively, since GAD and IA-2 proteins are present in various cell types, the long-term persistency of GADab and IA-2ab may reflect propensity to maintain autoimmunity once triggered, irrespective of the presence of B-cells.

The persistency of GADab and IA2ab allows retrospective diagnosis of autoimmune T1D in a large subset of patients with long-lasting diabetes. Both antibodies should be assayed since among our patients 10% were positive only for IA-2ab. Accurate diagnosis of T1D has clinical consequences. First, it should prompt to the search for other organ specific autoimmune diseases, as seen in 21% of our patients. Second, the persistency of detectable endogeneous insulin secretion has opened the way to intervention studies in patients with long-standing T1D [14]. In this respect, diabetes-associated autoantibodies and/or T-lymphocyte reactivity could be used to select patients who may benefit from such interventions [15]. Third, it may be difficult to differentiate on clinical grounds T1D from monogenic diabetes, particularly hepatocyte nuclear factor (HNF)1A- and HNF4A-maturity onset diabetes of the young (MODY) [2]. The diagnosis of MODY has consequences on treatment, family screening and screening for associated diseases. Particularly, the diagnosis of HNF1A- or HNF4A-MODY can lead to successful replacement of insulin therapy by sulfonylureas in patients initially misclassified as T1D [4]. However, genetic screening is costly and the pick-up rate for MODY is only 10–15% in reference laboratories [16]. Given the very low prevalence of diabetes-associated antibodies in patients with confirmed MODY, the absence of these antibodies could be a prerequisite for genetic testing [17].

In conclusion, GADab and/or IA-2ab are found in the majority of patients with T1D of more than 10-year duration. The long-term persistency of diabetes-associated antibodies, mainly GADab, is related to diabetes duration, to age at onset and to female sex. GADab and IA-2ab are useful tools to accurately classify patients even long after the onset of the disease.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

References


Table 4

Interaction of sex and DR3 on the presence of GAD and extrapancreatic antibodies in patients with type 1 diabetes.

<table>
<thead>
<tr>
<th></th>
<th>GADab+/EPA+</th>
<th>GADab+/EPA−</th>
<th>GADab−/EPA+</th>
<th>GADab−/EPA−</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

GADab: glutamic acid decarboxylase autoantibodies; EPA: extrapancreatic autoimmunity. Data are numbers with percentages into brackets.


