AUTOIMMUNE MARKERS IN SLOW TYPE 1 DIABETES: CONFRONTATION TO TYPE 1 DIABETES

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SUMMARY - Slow onset type 1 diabetes is an heterogeneous entity. Its clinical features may mimic type 2 diabetes but its pathophysiological mechanisms are close to type 1 diabetes.

Aim of the study: To find out the frequencies, levels and associations of ICA, GADab and IA-2ab in type 2 diabetic patients with atypical phenotype.

Patients and methods: ICA, GADab and IA-2ab were determined in: – 61 patients (age at diagnosis 48.2 ± 10, range 36-73 years) with an initial diagnosis of type 2 diabetes but having at least one symptom suggesting a slow type 1 diabetes (loss of weight, absence of obesity at diagnosis or secondary failure of oral hypoglycaemic agents). – 70 patients with type 1 diabetes (age18 ± 8.9, range 2-35 years). Clinical data evaluated in slow type 1 were maximal BMI, BMI and loss of weight at diagnosis and autoimmune disease. Fasting C-peptide or insulinemia.

Results: (Slow type 1 diabetes versus type 1 diabetes). ICA (43% vs 70%; p < 0.01) and IA-2ab (16% vs 75%; p < 0.01) were more frequent in type 1. GADab were as frequent (62% vs 74%). Association of the three antibodies (15.7% vs 58.5%; p < 0.05) were more frequent in type 1. Prevalence of GADab alone (27.5% vs 75%; p < 0.05) was higher in slow type 1 diabetes and with higher levels (median 55.5 UI/ml vs 17 UI/ml; p < 0.01). There was no difference for levels of ICA (25.5 UJDF/ml vs 28 UJDF/ml) or IA-2ab (11.5 UI/ml vs 38.5 UI/ml). BMI of GADab positive patients was lower. Delay of insulinotherapy was shorter in GADab or ICA positive patients. We did not find any relationship between antibodies presence and fasting C-peptide or insulinemia.

Conclusion: Slow type 1 diabetes should be evoked in atypical type 2 diabetes. Slow onset type 1 diabetic patients have different autoimmune patterns suggesting a different pathophysiological process. GADab and ICA are useful markers to predict future insulinopenia.

Key-words: slow type 1 diabetes, LADA, ICA, GAD antibodies, IA-2 antibodies.

 RéSUMÉ - Les marqueurs d’autoimmunité dans le diabète de type 1 lent : confrontation aux données du diabète de type 1.

Le diabète de type 1 lent est une entité hétérogène proche du diabète de type 2 par sa présentation clinique et du diabète de type 1 par ses mécanismes étiopathogéniques.

Objectifs : Déterminer la fréquence, les combinaisons et les taux des ICA, des Ac anti-GAD et anti-IA-2 chez des diabétiques classés type 2 mais de phénotype atypique et les comparer à une population de type 1.

Méthodes : La recherche des ICA, des Ac anti-GAD et anti-IA-2 a été réalisée chez : – 61 diabétiques de plus de 35 ans classés type 2 (âge moyen 48,2 ± 10 ans) ayant au moins une caractéristique évoquant un diabète de type 1 lent (amaigrissement ou poids normal au diagnostic, échappement secondaire à un traitement oral maximal), – 70 diabétiques de moins de 35 ans (âge moyen 18 ± 8,9 ans) ayant un diabète de type 1. Le BMI au diagnostic, le BMI maximal, l’amaigrissement, le terrain auto-immun, le délai d’insulinothérapie ainsi que le C-peptide et l’insulinémie IRMA à jeun ont été relevés dans le groupe de type 1 lent.

Résultats : (Type 1 lent versus type 1). Les ICA (43 % vs 70 %; p < 0,01) et les anti-IA-2 (16 % vs 75 %; p < 0,01) sont plus fréquents dans le type 1. Les anti-GAD sont retrouvés à la même fréquence (62 % vs 74 %; NS). Les combinaisons des 3 anticorps (15,7 % vs 58,5 %; p < 0,05) sont plus fréquentes dans le type 1. La présence isolée des anti-GAD (27,5 % vs 75 %; p < 0,05) est plus fréquente dans le type 1 lent et à des taux plus élevés (médiane de 55,5 UI/ml vs 17; p < 0,01). Il n’y a pas de différence significative pour la présence isolée (7,8 % vs 0 %) ni pour les taux (25,5 UJDF vs 28) des ICA ou des anti-IA-2 (0 % vs 8 %) et (11,5 UI/ml vs 38,5). Une relation négative existe entre la présence des anti-GAD et le BMI au diagnostic. Le délai d’insulinothérapie est plus court chez les patients anti-GAD+ ou IA-2+. Aucune relation n’est mise en évidence entre les Ac et le C-peptide ou l’insulinémie.

Conclusion : Le diabète de type 1 lent doit être évoqué devant un type 2 atypique. Les diabétiques de type 1 et de type 1 lent ont un profil d’auto-immunité humorale différent. Les anticorps anti-GAD et ICA constituent un outil diagnostique utile pour la prédiction de l’insulinopénie.

Mots-clés : diabète de type 1 lent, LADA, ICA, anticorps anti-GAD, anticorps anti-IA-2.

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Diabetes is an heterogeneous group of hyperglycaemic diseases within which the most frequent are type 1 and type 2 diabetes. These two diseases are distinct with respect to aetiology, pathogenesis and clinical course [1]. However, discrimination between them can be difficult at diagnosis, especially in adults. Indeed, diabetes presenting in adulthood has many faces and covers a broad spectrum of phenotypes. Immune-mediated diabetes commonly occurs in childhood and adolescence but it can occur at any age and then, often induces a slower deterioration in metabolic control [2]. Clinical manifestations in adult-onset type 1 diabetes are then less acute than in young patients and is so called slow-onset type 1 diabetes. It may mimic noninsulin dependent diabetes mellitus for several years, renders its discrimination difficult on clinical grounds and is more frequent than formerly believed [3]. Understanding aetiology and pathogenesis of diabetes is pointed out with the new classification of diabetes [1]. Type 1 diabetes is defined as a result of autoimmune β-cell destruction with the presence of immunological markers. Immunological analysis in type 1 diabetes is based on the detection of various autoantibodies such as islet-cell antibodies (ICAs), anti-glutamic acid decarboxylase (GADab), anti-insulin (IAA), anti-IA-2 (IA-2ab) and anti-IA-2β antibodies. Detection of antibodies is then one of the way to classify diabetes and to diagnose slow-onset type 1 diabetes in adults. So far slow type 1 diabetes also designated as LADA (latent autoimmune diabetes in adults) is not a well defined entity. Nevertheless, we know that in this form of diabetes, autoimmune β-cell destruction is slow and a good glycemic control can initially be obtained with oral drugs but it later progress to insulin dependency [4]. These patients are often initially diagnosed as type 2 diabetes. Type 2 diabetes is defined by the association of varying degrees of insulin resistance and insulin secretory defect [1]. The UKPDS showed that 10% of “type 2” diabetic patients were positive for GADab and 3% were positive for ICA [5] and some authors showed up to 20% of immune-mediated diabetes [6, 7]. These patients were more likely to progress to insulin dependency: 52% of GADab+ patients were treated with insulin after 6 years [5]. Autoimmune markers are then good predictors for future insulin dependence [7-9] and slow-onset type 1 diabetics could be suitable candidates for therapeutic strategies that seek to prevent progression to insulinopenia. Moreover, their discrimination could help avoiding a delay for insulin therapy [10].

The association of three antibodies, ICA, GADab and IA-2ab is now recognised as a suitable tool for prediction of type 1 diabetes. The purpose of this study was to compare the humoral pattern of atypical type 2 diabetes compared to juvenile type 1 diabetes. We have therefore investigated these three major antibodies and compared their frequencies, levels and associations in these two groups. We also studied the relationships between autoantibodies, clinical characteristics and the delay to insulin therapy in atypical type 2 diabetes.

**RESEARCH DESIGN AND METHODS**

**Patients** – The study group consisted of sixty-one diabetic patients (age at diagnosis 48.2 ± 10, median 47, range 36-73 years) admitted in the Departments of Endocrinology, University of Lille, between 1996 and 1998. Patients were included in the study when diabetes onset occurred after 35 years of age and type 2 diabetes was the initial diagnosis. They had at least one characteristic suggesting a slow type 1 diabetes such as a loss of weight > 5 kgs at diagnosis (22.9%), the absence of obesity at diagnosis with BMI < 27 (86.8%) or a secondary failure of oral hypoglycaemic agents with a delay > 6 months (36%). These patients did not exhibit symptoms of absolute insulin deficiency such as ketoacidosis. Ethnic distribution was: 49 Caucasians, 10 Caucasoids from Maghreb and 2 Asians. Sex-ratio was 32 females/29 males. This group as determined on clinical grounds will be named slow onset type 1 diabetes all along the article. Diabetes had been classified according to the previous NDGG criteria [11]. Mean diabetes duration was 4.9 ± 6.8 years (median 2, range 0-28 with 23 patients whose diabetes was diagnosed within 6 months) and 83.7% were treated with exogenous insulin. Patients with pancreatitis, steroids or immunosuppressive treatments were excluded. The assessed clinical data were maximal BMI, BMI at diagnosis, loss of weight at diagnosis, familial background of type 1 or type 2 diabetes and autoimmune diseases. This group was compared to a second group of 70 type 1 diabetic patients (duration of diabetes < 6 months, age 18 ± 8.9, median 17, range 2-35 years, sex ratio 26 females/44 males) also participating in the “12th International Histocompatibility Workshop Study” [12].

**Biological data** – Serum samples were collected in adults and fasting C-peptide (47.5% of cases) or fasting insulinaemia (46% of cases) were evaluated. Serum C-peptide concentrations were measured with a commercial radioimmunossay kit and expressed in nmol/l (RIA-coat C-peptide, Mallinckrodt, France) and inulinemia with an immunoradiometric assay and expressed in IU/l (Bi-Insulin IRMA, Sanofi Pasteur, France).

**Detection of cytoplasmic cell antibodies (ICA)** – Islet Cell Antibodies were detected by the indirect immunofluorescence test on cryostat sections of human pancreas from blood group O organ donors. Results are expressed in Juvenile Diabetes Foundation Units (UJDF). Antibody titers were determined by serial dilutions to end-point, measurements were performed.
according to the protocol from the International Workshop for the standardization of the ICA assay. Technical validity was assessed in the “13th ICA Proficiency Test” with a diagnostic sensitivity of 83% and a specificity of 75% for type 1 diabetes. The detection limit of our assay is 2.5 U/mL.

Detection of autoantibodies to glutamate decarboxylase (GADab) and to protein tyrosine phosphatase IA-2 (IA-2ab) – Autoantibodies to GAD and IA-2 were determined using radio-binding assay with labeled human recombinant proteins (Cis-Bio International, Oris Group, Gif-Sur-Yvette, France). The inter-assay and intra-assay coefficients of variation were 5.1% and 4.3% for anti-GAD and 3.4% and 4.3% for anti-IA-2. The detection limit was 0.196 UI for anti-GAD and 0.5 UI for anti-IA-2. The GADab assay was evaluated in 1998-1999 in the 4th International Proficiency GAD Study conducted by Dr N.K MacLaren (University of Louisiane) and showed a sensitivity and specificity of 100%. The cut-off value of 1U/ml represents the mean ± 3 SD of the GADab values obtained in 100 healthy school children (age: 11±2 years). Regarding IA-2ab, the cut off of 1 U/ml was used according to manufacturers’ instructions. Antibodies to insulin were not investigated because of the high number of patients already treated with exogenous insulin.

Delay of antibodies detection – Antibodies were assessed when patients were admitted in our Department: delay of antibodies detection was then the same as duration of diabetes which was 4.9 ± 6.8 years (median range 0-28 with 23 patients whose diabetes was diagnosed within 6 months). Because of the varying duration of diabetes at the time of antibodies detection in type 2 diabetic group, we checked for the absence of relation between diabetes duration, levels and prevalence of antibodies with a correlation test.

Statistical analysis – Values are given as mean ± SD. The significance of differences between frequencies was tested using the chi-square test with Fisher’s correction. Fisher’s test, Kruskall-Wallis or Wilcoxon tests were used when appropriate. Pearson correlation coefficient was used to assess significance between antibodies levels and clinical or biological patients’ characteristics. Relation between autoantibodies and the initiation of insulin treatment was tested with a survival analysis. p < 0.05 was significant. Statistical tests were performed by the University Statistical Department (CERIM, LILLE II, France).

■ RESULTS

Frequency, association and levels of ICA, GADab and IA-2ab in slow onset and type 1 diabetes

Frequency of antibodies (Fig. 1) – Both ICA and IA-2ab were significantly less frequent in slow onset type 1 diabetes than in type 1 diabetes (43% vs 70%, p < 0.01 and 16% vs 75%, p < 0.01, respectively). GADab were as frequent in slow onset as in type 1 diabetes (62% vs 74%, respectively, NS).

Association of the 3 antibodies ICA, GADab and IA-2ab (Fig. 2) – Association of the 3 antibodies was significantly less frequent in slow onset type 1 diabetes than in type 1 diabetes (15.7% vs 58.5%, p < 0.05). Prevalence of GADab alone was significantly higher in slow onset diabetes (27.5%) than in type 1 diabetes (7.5%, p < 0.05). Prevalences of ICA or IA-2ab alone were not significantly different between the two groups. Noteworthy there was no patient with ICA alone in type 1 diabetes group and 7.8% in slow onset group.

Levels of antibodies (Fig. 3, 4 et 5) – Levels of GADab (Fig. 3) were significantly higher in slow onset type 1 diabetes compared to type 1 diabetes (median range 55.5 vs 17, respectively, p < 0.01). There was no significant differences regarding ICA levels (Fig. 4) (median range 25.5 vs 28 respectively,
p = 0.8) and anti-IA-2 levels (Fig. 5) although the latter tended to be higher in type 1 diabetes (median range 11.5 vs 38.5, respectively, p = 0.4)

Clinical and biological characteristics of slow onset diabetic patients

Phenotype at diagnosis (Table I) – Clinical characteristics of slow onset type 1 patients are detailed in

Table I according to GADab positivity. Ketonuria, presence of hypertension or long-term complications were not different between the two groups. There was also no differences regarding familial or personal autoimmune background.

Clinical characteristics and antibodies – GADab positivity was associated with symptoms at diagnosis (28% vs 9%, p < 0.05) and levels expressed as mean (median) then were higher: 56.5 IU/ml (20.5) vs 29.7 IU/ml (0), p < 0.05. BMI at diagnosis was significantly higher in GADab negative patients than in GADab positive patients but with a great overlap between the two groups. BMI seemed to be more variable in GADab positive patients (Fig. 6). GADab positive patients lost weight more frequently at diagnosis however the loss of weight in kilograms tended to be higher but was not significantly different (p = 0.058). GADab positivity was not more frequent in patients with autoimmunity (thyroiditis, Basedow, vitiligo...) but levels were then higher: 105.5 IU/ml (118) vs 65.5 IU/ml (32.7), p = 0.01. Patients with autoimmunity had IA-2 ab more frequently (55% vs 15%, p < 0.05) but IA-2 ab levels were then lower in these patients: 7.5 IU/ml (6.6) vs 25.7 UI/ml (15.2), p=0.01. There was no relation between ICA and autoimmunity.

Antibodies, insulinoserection and delay of insulino-therapy – Fasting C-peptide and fasting insulinenia were not significantly related to the presence of GADab although there was a tendency for C-peptide (p = 0.054). Fasting plasma glucose values were not different between patients with or without GADab:
2.66 g/l (2.67) vs 2.61 g/l (2.76). Survival analysis was used to test the relationship between the presence of antibodies and the delay before insulin therapy: there was a relation between ICA (p < 0.05), GADab (p < 0.01) but not IA-2ab (p = 0.08) and the initiation of insulin therapy.

## DISCUSSION

We compared the prevalence of type 1 associated antibodies in two groups of patients: in diagnosed type 2 diabetic patients having at least one characteristic suggesting a slow type 1 diabetes and acute type 1 diabetic patients. We showed that distinct immunological patterns discriminate slow type 1 from acute type 1 diabetes. These differences regard the prevalence, the levels of each antibody and their combination to each other. Particularly, the slow type 1 diabetic group was characterised by higher levels, higher prevalence of isolated GADab and less frequent association between the 3 antibodies.

The high frequency of GADab in slow onset type 1 diabetic patients has already been described but higher levels are less emphasized [13]. Firstly, there could be a kinetic reason. GADab persist for years even if they occur very early, preceding ICA and IA-2ab in children [14, 15]. GADab and ICA persist longer in slow type 1 diabetes [16-19], ICA decreasing more rapidly than GADab which persist until 20 years after onset of slow type 1 [8, 15, 20-23]. These results could suggest that ICA and IA-2ab are present long before diagnosis of slow type 1 diabetes and already decreased at diagnosis [24]. Our results are to be interpreted with caution because of the varying delay of investigation. However, IA-2ab are negative even when in case of an impaired glucose tolerance only [25, 26]. The second hypothesis is that GADab reflects a cross reaction with GAD-related epitopes expressed by other endocrine cells [27, 28] which then could stimulate non specific humoral immunity [29]. This hypothesis might account for the higher levels of GADab found in patients with autoimmunity as already described in diabetic patients with thyroid disease [28].

<table>
<thead>
<tr>
<th>positive GAD ab</th>
<th>negative GAD ab</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex ratio (F/H)</td>
<td>21/14</td>
<td>10/13 NS</td>
</tr>
<tr>
<td>age at diagnosis</td>
<td>47.5 (45)*</td>
<td>47.9 (46) NS</td>
</tr>
<tr>
<td>diabetes duration (months)</td>
<td>62.5 (30)</td>
<td>60.5 (24) NS</td>
</tr>
<tr>
<td>familial background of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>– type 2 diabetes</td>
<td>22.8 %</td>
<td>26 % NS</td>
</tr>
<tr>
<td>– type 1 diabetes</td>
<td>11.4 %</td>
<td>21.7 % NS</td>
</tr>
<tr>
<td>– both</td>
<td>2.8 %</td>
<td>8.7 % NS</td>
</tr>
<tr>
<td>auto-immune disease</td>
<td>25.7 %</td>
<td>8.7 % NS</td>
</tr>
<tr>
<td>maximal BMI</td>
<td>26.9 (25.5)</td>
<td>27.5 (26) NS</td>
</tr>
<tr>
<td>range of maximal BMI</td>
<td>19-41</td>
<td>21-42 NS</td>
</tr>
<tr>
<td>BMI at diagnosis</td>
<td>23.1 (23)*</td>
<td>24.8 (25)* &lt; 0.05</td>
</tr>
<tr>
<td>range of BMI at diagnosis</td>
<td>16-33</td>
<td>19-28</td>
</tr>
<tr>
<td>loss of weight</td>
<td>77 %</td>
<td>47.8 % &lt; 0.05</td>
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<tr>
<td>loss of weight (kgs)</td>
<td>5.5 (5)*</td>
<td>3.3 (0)* 0.058</td>
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<td>ketonuria at diagnosis</td>
<td>28.5 %</td>
<td>26 % NS</td>
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<td>HTA at admission</td>
<td>28 %</td>
<td>30.4 % NS</td>
</tr>
<tr>
<td>complications at admission</td>
<td>31.4 %</td>
<td>39 % NS</td>
</tr>
</tbody>
</table>

*: results are mean (median). †: diabetic retinopathy, nephropathy, neuropathy or macrovascular disease.
question is the paradoxical link between insulin secretion and GADab. Immunological studies showed an inverse relationship between cellular (predictive of β-cell destruction) and humoral (predictive of β-cell preservation) immunity to GAD [30]. Indeed high levels of GADab are associated with a better insulin secretion in children [31]. Paradoxically, presence but also levels of GADab have been described as markers of future insulinopenia in adults [5, 13, 32-36]. Slow type 1 patients may constitute an intermediate population between type 1 and type 2 diabetes characterized by insulin resistance but with a more severe defect in stimulated β-cell capacity than type 2 diabetic patients as shown recently [37]. Because of GADab preponderance, some studies have suggested that testing GADab without ICA detection may be sufficient to diagnose slow onset type 1 diabetes [38]. Indeed GAD [39] and IA-2 [40] have been identified as the major ICA antigens. However, combined detection of ICA and GADab improves the predictive value [5, 19]. In our group, ICA alone were detected in 7.8% of slow onset patients as shown by Seissler et al. [41]. This could argue for a restriction of ICA to unknown antigens in slow type 1 diabetes [25, 41, 42]. Indeed, IA-2ab are less frequent in slow type 1 [38, 41] than in type 1 diabetes [42]. Our data show a IA-2ab prevalence of 16.4% compared to 6% in literature. This relatively high frequency may be due to selection on clinical criteria compared to systematic detection in type 2 diabetes in the literature. Diagnosis criteria for slow type 1 diabetes are not well defined and it appears as a subtype of type 1 diabetes in the expert committee report [1]. Different authors have proposed criteria but with no consensus [13, 18, 38, 41]. The clinical and biological characteristics of our patients were studied according to their antibody status. We found differences for loss of weight and BMI at diagnosis. The great overlap for the patients weight between our two groups confirm that BMI is not a good individual predictor of slow onset type 1 diabetes even if Groop and al have described patients with GADab as younger, less overweight and android than other.
type 2 diabetic patients [38]. GADab positive patients in our study tend to have lower fasting C-peptide and insulin as shown by Groop. There was an overlap between the two groups regarding C-peptide. Then, it seems that dynamic tests on insulin or C-peptide would be better to assess insulin secretion and discriminate slow type 1 diabetic patients as shown recently [37]. Besides we did find a correlation between the presence of ICA and/or GADab (but not IA-2ab) with the delay before insulin therapy [43]. Then we looked for an association between antibodies and auto-immune diseases. Our data differed from a previous analysis of type 1 diabetes and thyroid immunity where higher levels and prevalence of GADab were reported with no differences for IA-2ab [44]. All these observations lead us to conclude that immunity in diabetes is heterogeneous, suggesting various pathophysiological processes. Is it a unique and different disease entity? Genetic studies of slow type 1 diabetes described different pattern compared to type 1 diabetes [38] and further investigations are clearly needed. GADab are superior to other antibodies in the recognition of slow type 1 diabetes [13] but their predictive value may increase when combined with ICA even if ICA detection is a matter of debate in the literature [38, 41]. In clinical practice, slow onset type 1 diabetes should systematically be evoked in non typical type 2 diabetes. Another still debated issue is the treatment to be proposed to these patients. A prospective study comparing the benefits of insulin therapy versus oral agents would be useful to answer to this question.

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