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Differences in prevalence of antibodies to GAD and IA-2 and their titers at diagnosis in children with slowly and rapidly progressive forms of type 1 diabetes

Tatsuhiko Urakami *, *Ayako Yoshida*, *Junichi Suzuki*, *Hiroshi Saito*, *Mika Wada*, *Shouri Takahashi*, *Hideo Mugishima*

Department of Pediatrics, Nihon University School of Medicine, 1-8-13 Kandasurugadai Chiyoda-ku, Tokyo 101-8309, Japan

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ABSTRACT

We compared the frequencies of antibodies to GAD (GADA) and IA-2 (IA-2A) and their titers at diagnosis in 48 Japanese children with slowly progressive form of type 1 diabetes (SPT1D) and 70 children with rapidly progressive form of type 1 diabetes (RPT1D). High prevalences of both GADA and IA-2A were found at diagnosis in both the patients with SPT1D (70.8% and 75.0%), and those with RPT1D (71.4% and 71.9%). Most patients, regardless of the form of type 1 diabetes, were positive for both antibodies, though 6 of the 9 patients less than 5 years of age were negative for both antibodies. GADA titers below 50 U/ml were significantly more frequent in the patients with SPT1D (79.4% vs. 38.0%, $p = 0.0002$), and titers above 100 U/ml significantly more frequent in those with RPT1D (38.0% vs. 11.8%, $p = 0.0081$). No significant association was noted between the titers of IA-2A and the clinical form of type 1 diabetes. These results suggest that low GADA titers may reflect mild autoimmune destruction of beta-cells with slow disease progression. Titers of IA-2A do not appear to reflect the degree of autoimmune damage of the beta-cells.

1. Introduction

The rate of destruction of pancreatic beta-cells during the course of type 1 diabetes is quite variable, being rapid in some individuals and slow in others. Clinical characteristics of the slowly progressive form were initially described by Kobayashi et al. in Japan [1,2]. Patients with this form of diabetes may retain residual beta-cell function during the early stage of the disease, sufficient to prevent metabolic deterioration for several months or even years. However, in the later stages of this disease, there is little or no insulin secretion, as manifested by low or undetectable plasma levels of C-peptide [3,4], and patients eventually become dependent on insulin treatment for maintenance of adequate glycemic control. This form of diabetes generally occurs in adults and has been referred to as latent autoimmune diabetes in adults (LADA) in Caucasian populations [5,6]. The clinical features of the slowly progressive form of type 1 diabetes in children were introduced by our group [7,8]. Most Japanese children with this form of type 1 diabetes are detected through the urine glucose screening program conducted at schools, along with the many type 2 diabetes cases detected, who have only moderate symptoms of diabetes [9,10].

* *Corresponding author*. Tel.: +81 3 3293 1711; fax: +81 3 3293 1711.
E-mail address: turakami@med.nihon-u.ac.jp (T. Urakami).

Abbreviations: GADA, antibody to glutamic acid decarboxylase; IA-2A, antibody to protein tyrosine phosphatase IA-2; ICA, islet-cell antibodies; LADA, latent autoimmune diabetes in adults; OGTT, oral glucose tolerance test; RPT1D, rapidly progressive form of type 1 diabetes; SPT1D, slowly progressive form of type 1 diabetes.

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We have demonstrated that children with the slowly progressive form of type 1 diabetes (SPT1D) have a high prevalence of islet cell antibodies (ICA) at diagnosis, similar to cases with the rapidly progressive form of type 1 diabetes (RPT1D), and that ICA tends to persist with low antibody-titers for relatively long periods of time after the diagnosis. This may reflect slow disease progression caused by slow autoimmune destruction of insulin-secreting beta-cells in children with SPT1D [8].

In the present study, we compared the prevalences and patterns of elevation of antibodies to glutamic acid decarboxylase (GADA) and protein tyrosine phosphatase IA-2 (IA-2A), which are known to be immune markers in sera for type 1 diabetes, differing from ICA, at diagnosis in children with SPT1D and RPT1D to elucidate the possible roles of these two antibodies in beta-cell autoimmunity.

2. Materials and methods

2.1. Patients

Forty-eight Japanese children, 14 males and 34 females, with a mean age of 11.6 ± 2.4 (6.8–15.8) years, diagnosed as having SPT1D were studied. These children were diagnosed based on the results of the urine glucose screening program conducted at schools in the Tokyo metropolitan area during the period from 1974 to 2005. Of these 48 children, 47 (98%) were non-obese with a body mass index of less than 22 at diagnosis. They maintained relatively high serum levels of C-peptide, sufficient to prevent ketosis within 15 months after the diagnosis. Some did not require insulin treatment during the early stage of the disease. Thereafter, endogenous insulin secretion gradually decreased and they required insulin treatment for adequate glycemic control [7,8]. There were no cases with genetic abnormalities including maturity-onset diabetes of the young and mitochondrial diabetes.

Meanwhile, patients with RPT1D, consisting of 34 males and 36 females, with a mean age of 8.1 ± 3.4 (1.0–13.4) years, exhibited typical symptoms of diabetes and/or ketosis at diagnosis. All patients required insulin supplementation for survival and/or to manage metabolic deterioration after the diagnosis.

We have conducted an annual urine screening program for school-age children, 6–15 years old, residing in the Tokyo metropolitan area for glucosuria concomitant with proteinuria and hematuria, since 1974. The annual rate of participation in the urine screening program has been nearly 100%. Urinalysis was carried out on morning urine specimens using glucose oxidase strips. If a urine sample test was positive, a subsequent urine test was requested on another morning. An oral glucose tolerance test (OGTT) was performed when positive results were obtained on both the initial and the second urine tests to confirm the diagnosis of diabetes. For the OGTT, 1.75 g/kg (maximum 75 g) of glucose was used, and the U.S. Public Health Service criteria and/or the World Health Organization criteria for the diagnosis of glucose intolerance were adopted. HbA1c levels, serum insulin concentrations, urinary ketone bodies, etc. were also examined to confirm the diagnosis of diabetes. Most of the children whose diabetes was detected by the screening program were identified as having type 2 diabetes [9,10].

2.2. Methods

We examined the prevalences of GADA and IA-2A and their titers at diagnosis in patients with SPT1D, and compared the results to those of RPT1D cases. The prevalences and titers of GADA were examined in 48 children with SPT1D and 70 with RPT1D. Those of IA-2A were examined in 28 children with SPT1D and 57 with RPT1D. All subjects analyzed for IA-2A titers were also examined for GADA using the same serum samples.

Serum samples were collected from the patients at diagnosis and stored at $-70\,^{\circ}C$ until the antibody-determinations were performed. GADA and IA-2A were determined by radioimmunoassay (Cosmic Corporation, Tokyo, Japan). The cut-off limits for GADA and IA-2A antibody positivity were 1.5 U/ml and 0.4 U/ml, respectively.

2.3. Statistical analysis

To detect differences in frequencies between two groups, Chi-square test was applied and $p < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. Prevalences of GADA and IA-2A at the time of diagnosis

The overall prevalences of GADA and IA-2A at diagnosis in the children with SPT1D were 70.8% (34/48) and 75.0% (21/28), respectively. The corresponding prevalences in those with RPT1D were 71.4% (50/70) and 71.9% (41/57). Thus, there were no statistically differences in the prevalences of the two types of antibodies at diagnosis between the children with SPT1D and those with RPT1D.

Among the children with RPT1D who were younger than 5 years at diagnosis (1.0–4.8 years of age), the prevalence of GADA was 31.6% (6/19) and that of IA2-A was 47.1% (8/17). On the other hand, among those with RPT1D in whom the age at onset was beyond 5 years, the prevalence of GADA was 86.3% (44/51), that of IA-2A 82.5% (33/40). Thus, the prevalences of both GADA and IA-2A were significantly lower in patients who were younger at onset ($p < 0.0001$ and $p = 0.0064$, respectively). In contrast, there was no significant association between the prevalences of the two types of antibodies and the age at onset in children with SPT1D.

3.2. Prevalence of the combined presence of GADA and IA-2A at the time of diagnosis

The prevalence of the combined presence of GADA and IA-2A at diagnosis was also analyzed in the two groups of children with type 1 diabetes. Among the 28 children with SPT1D analyzed for the two types of antibodies, 21 (75.0%) had both GADA and IA-2A, 3 (10.7%) had GADA alone, and 4 (14.3%) neither GADA nor IA-2A. None of the patients with SPT1D were

Table 1 – Prevalences of GADA and IA-2A at the time of diagnosis in children with SPT1D and RPT1D.

	SPT1D	RPT1D	p^*
GADA	34/48 (70.8%)	50/70 (71.4%)	>0.9999
IA-2A	21/28 (75.0%)	41/57 (71.9%)	0.9659
GADA+/IA-2A+	21/28 (75.0%)	34/57 (59.6%)	0.2488
GADA+/IA-2A−	3/28 (10.7%)	6/57 (10.5%)	>0.9999
GADA−/IA-2A+	0	8/57 (14.0%)	0.0906
GADA−/IA-2A−	4/28 (14.3%)	9/57 (15.8%)	>0.9999

(+) Positive for the antibody; (−) negative for the antibody.
* SPT1D vs. RPT1D.

positive for IA-2A alone. On the other hand, among the 57 children with RPT1D, 34 (59.6%) had both GADA and IA-2A, 6 (10.5%) had GADA alone, 8 (14.0%) had IA-2A alone, and 9 (15.8%) were negative for both. There were no statistically differences in the frequencies of the combined presence of GADA and IA-2A at diagnosis between the children with SPT1D and those with RPT1D. Interestingly, 6 of the 8 patients who were positive for IA-2A alone and 6 of the 9 who were negative for both, among the RPT1D patients, had been diagnosed with diabetes before 5 years of age (Table 1).

3.3. *Distributions of the titers of GADA and IA-2A at the time of diagnosis*

Table 2 presents the distributions of the titers of GADA and IA-2A at diagnosis in children with the two forms of type 1 diabetes.

Patients with SPT1D had lower GADA titers, while those with RPT1D had higher titers, i.e. GADA titers of less than 50 U/ml were significantly more frequent in children with SPT1D (79.4%) than in those with RPT1D (38.0%) ($p = 0.0002$). On the other hand, GADA titers exceeding 100 U/ml were significantly more frequent in children with RPT1D (38.0%) than in those with SPT1D (11.8%) ($p = 0.0081$).

Lower IA-2A titers were relatively more common in both forms of type 1 diabetes, i.e. the frequencies in IA-2A titers below 10 U/ml were 76.2% in children with SPT1D and 60.9% in those in RPT1D. These figures differed from the corresponding percentages with relatively high titers ($p = 0.0007$ in SPT1D, $p = 0.0468$ in RPT1D). There was no significant relation between the changes in the titers of IA-2A and the clinical forms of diabetes.

Table 2 – Titers of GADA and IA-2A at the time of diagnosis in children with SPT1D and RPT1D.

	SPT1D	RPT1D	p^*
Titers of GADA (U/ml)			
10>	17/34 (50.0%)	10/50 (20.0%)	0.0080
10–50	10/34 (29.4%)	9/50 (18.0%)	0.3363
<50	27/34 (79.4%)	19/50 (38.0%)	0.0002
50–100	3/34 (8.8%)	12/50 (24.0%)	0.1356
100–200	2/34 (5.9%)	7/50 (14.0%)	0.4114
200	<2/34 (5.9%)	12/50 (24.0%)	0.0589
>100	4/34 (11.0%)	19/50 (38.0%)	0.0081
Titers of IA-2A (U/ml)			
5	>8/21 (38.1%)	14/41 (34.1%)	0.9783
5–10	8/21 (38.1%)	11/41 (26.8%)	0.5355
10–20	4/21 (19.0%)	9/41 (22.0%)	>0.9999
20	>1/21 (4.8%)	7/41 (17.1%)	0.3329

* SPT1D vs. RPT1D.

4. Discussion

SPT1D is considered to be a subtype of type 1 diabetes clinically distinct from RPT1D. This form of diabetes was initially reported in adult patients with immune markers of type 1 diabetes who did not require insulin in the early stage after the diagnosis, but eventually became insulin-dependent with gradual progression of the disease [1,2]. It has now been established that this form of diabetes is not limited to adults, and can also occur in children and adolescents [7,8,11,12].

We have demonstrated that Japanese children with SPT1D show largely preserved beta-cell function, as evaluated by serum C-peptide levels, for at least 2 years after the clinical onset of this disease [7]. Their ICA prevalence at diagnosis is high, 82%, and ICA tends to persist for many years at low titers of 20–40 JDF units [8]. We consider this observation to possibly reflect the slow progression of autoimmune damage to beta-cells. Other studies have also demonstrated a high prevalence of ICA at diagnosis in both adults [2,6,13] and children with SPT1D [11], though there were no significant differences in these titers as compared to those in patients with RPT1D.

In the present study, children with SPT1D had a high prevalence (70.8%) of GADA and IA-2A at diagnosis, similar to those with RPTID (71.4%). GADA is reported to be present in approximately 60–80% of Japanese subjects with newly diagnosed type 1 diabetes, the prevalence being similar to that reported in Caucasian patients, regardless of the clinical form of diabetes [14–16]. Yamada et al. reported a lower prevalence of GADA in young patients less than 5 years of age at the disease onset as compared with those who were 13–19 years of age at disease onset [17]. On the other hand, fewer subjects with newly diagnosed type 1 diabetes (32–75%) have been reported to be positive for IA-2A [14,15,18,19]. Several studies have demonstrated a decreased prevalence of IA-2A with increasing age at disease onset [20–22]. These findings are common to Japanese and Caucasian patients. In regard to the clinical forms of diabetes, some Japanese studies have indicated a lower prevalence of IA-2A in SPT1D [20,23]. The high prevalence of IA-2A in both of the clinical forms of diabetes in the present study may be attributable to the early age at diagnosis of our study subjects, and to the exclusion of subjects over 16 years of onset-age.

We found lower prevalences of both GADA and IA-2A among children with an onset age younger than 5 years. Moreover, prevalences of the absence of either or both type of autoantibody were higher in the children who were younger at disease onset. Several studies have reported a lower prevalence of beta-cell antibodies in younger children. Hathout et al. [24,25] described young children, i.e. those diagnosed prior to 5 years of age, as having significantly lower frequencies of ICA, GADA and IA-2A at diagnosis than older children. Furthermore, they demonstrated a higher incidence of viral illness prior to the onset of type 1 diabetes in young

children. Non-autoimmune diabetogenic mechanisms may also play a role in young children.

The combined presence of multiple beta-cell antibodies in adults with SPT1D has been analyzed in several studies. Hosszufalusi et al. [13] demonstrated that single-autoantibody positivity (ICA or GADA) was more often seen in adults with SPT1D than in those with RPT1D. Several studies have also indicated the presence of two or three beta-cell antibodies (ICA, GADA and IA-2A) at diagnosis to be predictive of severe deterioration of beta-cell function within a few years, whereas that of either GADA or IA-2A alone is associated with slower progression of the disease [18,22,23]. In the present study, the majority of patients were positive for both GADA and IA-2A regardless of the clinical form of type 1 diabetes. Moreover, patients with SPT1D had a higher frequency of simultaneous presence of both antibodies as compared to those with RPT1D (75.0% vs. 59.6%). Given these findings, children with SPT1D might more frequently be positive for multiple beta-cell antibodies than adults with this disease. Neither multiple nor single autoantibody-positivity is a predictive marker for disease activity in childhood type 1 diabetes. A more severe destruction of beta-cells at the time of clinical onset might present in childhood cases, even those with SPT1D.

We previously reported that children with SPT1D had lower titers of ICA at diagnosis than those with RPT1D [8]. In the present study, SPT1D was also found to be characterized by low titers of GADA. Some studies have also reported low serum concentrations of GADA in adult patients with SPT1D [13,26]. There seems to be a linkage between fluctuating titers of GADA and the disease activity [8,13,26]. On the other hand, lower IA-2A titers were more common in both forms of type 1 diabetes and no significant association between the changes in the titers of IA-2A and disease activity was found in the present study.

In conclusion, children with SPT1D had high prevalences of GADA and IA-2A at diagnosis, similar to children with RPT1D. Most children with SPT1D initially had both antibodies, with low titers of GADA. It is possible that low titers of GADA reflect mild autoimmune destruction of beta-cells with slow disease progression; in contrast, titers of IA-2A do not appear to reflect the degree of autoimmune damage of the beta-cells. Some immunological markers could reflect the clinical course of childhood type 1 diabetes.

Conflict of interest

There are no conflicts of interest.

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