JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Different interaction of onset age and duration of type 1 diabetes on the dynamics of autoantibodies to insulinoma-associated antigen-2 and zinc transporter 8

Eiji Kawasaki[1]*, Yoichi Oikawa[2,3], Akira Okada[4], Norio Kanatsuna[5], Tomoyuki Kawamura[6], Tadashi Kikuchi[7], Jungo Terasaki[5], Junnosuke Miura[8], Yoshihisa Itoh[9], Toshiaki Hanafusa[10]

[1]Diabetes Center, Shin-Koga Hospital, Kurume, Japan, [2]Department of Internal Medicine, Tokyo Saiseikai Central Hospital, Tokyo, Japan, [3]Department of Endocrinology and Diabetes, School of Medicine, Saitama Medical University, Saitama, Japan, [4]Okada Clinic, Fukuoka, Japan, [5]Department of Internal Medicine (I), Osaka Medical College, Takatsuki, Japan, [6]Department of Pediatrics, Osaka City University Graduate School of Medicine, Osaka, Japan, [7]Cosmic Corporation, Tokyo, Japan, [8]Diabetes Center, Tokyo Women's Medical University School of Medicine, Tokyo, Japan, [9]Eiju General Hospital, Tokyo, Japan, and [10]Sakai City Medical Center, Sakai, Japan

Keywords
Age, Autoantibodies, Type 1 diabetes

***Correspondence**
Eiji Kawasaki
Tel.: +81-942-38-2222
Fax: +81-942-38-2248
E-mail address:
e-kawasaki@tenjinkai.or.jp

J Diabetes Investig 2021; 12: 510–515

doi: 10.1111/jdi.13370

ABSTRACT

Aims/Introduction: This study aimed to investigate the dynamics associated with autoantibodies to insulinoma-associated antigen-2 (IA-2A) and zinc transporter 8 (ZnT8A) relating to the onset age and disease duration in patients with type 1 diabetes.

Methods: Using bridging-type enzyme-linked immunosorbent assay, IA-2A, ZnT8A and glutamic acid decarboxylase autoantibodies were evaluated in 269 patients with type 1 diabetes (median onset age 18.2 years, range 0.8–86 years; median diabetes duration 7 years, range 0–58 years). We then compared the prevalence of these autoantibodies among the different age groups, along with the duration of diabetes using the Cochran–Armitage trend test and multivariate logistic regression analysis.

Results: The prevalence of IA-2A, ZnT8A and glutamic acid decarboxylase autoantibodies in patients with duration of ≤3 years was 41.1, 36.7 and 72.2%, respectively, with 80.0% expressing one or more of these autoantibodies. This prevalence declined according to the disease duration ($P < 0.005$). Both IA-2A and ZnT8A were more frequently observed in younger patients, whereas glutamic acid decarboxylase autoantibodies was more common in older patients. Multivariate logistic regression analysis showed that there was a significant interaction between the onset age and duration of diabetes in patients diagnosed when aged ≤10 years regarding all anti-islet autoantibodies ($P < 0.05$). However, for patients diagnosed in the middle tertile (aged 11–30 years), the interaction was significant only for ZnT8A, and for those with late-onset diabetes (aged ≥31 years) only for IA-2A.

Conclusions: The current study showed that the rate of disappearance of anti-islet autoantibodies is faster in patients aged ≤10 years, and that even though both proteins are localized in the insulin granule membrane, humoral autoimmunity to IA-2 and ZnT8 differs according to the age of onset.

INTRODUCTION

Anti-islet autoantibodies are important serological markers for classifying individuals with diabetes. Previous reports state that at the onset of type 1 diabetes, one or more of the anti-islet autoantibodies, including autoantibodies to glutamic acid decarboxylase (GADA), insulin, insulinoma-associated antigen-2 (IA-2A) and zinc transporter 8 (ZnT8A), is detected in 95% of patients with type 1 diabetes[1,2]. In general practice, anti-islet autoantibody measurement is recommended as a routine clinical examination to distinguish autoimmune diabetes or to clarify diagnosis when physicians encounter "atypical" cases[3,4]. It is well known that Japanese patients with type 1 diabetes are

Received 18 May 2020; revised 22 June 2020; accepted 16 July 2020

classified into three subtypes depending on the manner of onset and progression; fulminant, acute-onset or slowly progressive type 1 diabetes[5]. Among these subtypes, the presence of anti-islet autoantibodies is essential in diagnosing acute-onset and slowly progressive type 1 diabetes. Due to anti-islet autoantibodies' tendency to disappear years after the emergence of diabetes, a possible diagnosis of type 1 diabetes should not be dismissed in long-standing diabetes, even in the absence of autoantibodies. Furthermore, the patient's age at onset also influences the prevalence of some anti-islet autoantibodies, and it has been reported that IA-2A and ZnT8A are more common in younger individuals[6–8]. However, despite compelling evidence, the interaction between age and duration of diabetes is still unresolved. To explore this question, we investigated how the age of onset and duration of diabetes relates to the prevalence of IA-2A and ZnT8A in contradistinction to GADA.

METHODS

Participants

A total of 333 serum samples from type 1 diabetic patients were collected from six contributing institutions for the present study. All patient samples were taken from an ethnically homogeneous Japanese population. We excluded a total of 64 patients, 27 diagnosed with fulminant type 1 diabetes, and a further 37 complicated by autoimmune thyroid diseases as a result of their anti-islet autoantibodies often persistent presence in comparison with cases solely involving type 1 diabetes[9]. Data for the remaining 269 patients, including 213 acute-onset type 1 diabetes and 56 slowly progressive type 1 diabetes, were used in all subsequent analyses. A diagnosis of type 1 diabetes was made based on the criteria set by the Committee of the Japan Diabetes Society[10–12]. The study protocols were approved by the ethics committee of each contributing institute, and informed consent was obtained from all participants. The clinical and immunological profiles of included and excluded patients are summarized in Table S1. Serum samples were stored at −20°C until use.

Anti-islet autoantibody measurement

Anti-islet autoantibodies (GADA, IA-2A and ZnT8A) were determined using a bridging-type enzyme-linked immunosorbent assay (RSR Ltd., Cardiff, UK), which uses the sandwich-type principle (bivalent enzyme-linked immunosorbent assay using biotinylated antigen), as previously described[13]. All results were read from a calibration curve constructed in the same run as the calibrators and expressed in U/mL. The cut-off value for the IA-2A was 0.6 U/mL, 10.0 U/mL for the ZnT8A and 5.0 U/mL for the GADA, respectively. The intra- and interassay coefficients of variation for the IA-2A were 2.0–3.3% and 4.0–6.5%, respectively, 3.5–6.2% and 7.5–9.3% for ZnT8A, and 3.5–8.5% and 5.2–6.4% for GADA (taken from the manufacturer's data sheets of the three kits; RSR Ltd.). In the 2018 Islet Autoantibody Standardization Program Workshop (Lab ID: 1801), the assay sensitivities and specificities achieved were 60% and 100% for IA-2A, 72% and 96% for ZnT8A, and 78% and 99% for GADA, respectively.

Statistical analysis

All results are expressed as the mean ± standard deviation or median (range), and the autoantibody prevalence was compared using the χ^2-test, Fisher's exact test and Cochran–Armitage's test where appropriate. Differences in non-parametric data were then tested using the Mann–Whitney *U*-test. The age of onset and duration of diabetes were categorized in tertiles: diabetes onset at 0–10 years, 11–30 years, and older than 31 years; duration of diabetes 0–3 years, 4–15 years, and more than 16 years. The association of autoantibody positivity with age and duration of diabetes were estimated using multivariate logistic regression models, and *P*-values <0.05 were considered statistically significant. Statistical analysis for this study was carried out using StatView statistical software (version 5.0; SAS Institute, Cary, NC, USA).

RESULTS

Relationship between age at onset and the prevalence of anti-islet autoantibodies

Of 85 patients diagnosed with type 1 diabetes aged ≤10 years, 35 (41.2%) and 29 (34.1%) were positive for IA-2A and ZnT8A, respectively. Autoantibody prevalence decreased with increasing age for both IA-2A and ZnT8A, whereas GADA became more prevalent in patients who were older at the time of diagnosis, with 74.7% of those aged ≥31 years testing positive (Table 1). Consequently, the prevalence of patients positive for one or more of these three autoantibodies was relatively stable among all age tertiles.

Relationship between duration of diabetes and the prevalence of anti-islet autoantibodies

As shown in Table 1, 37 (41.1%) and 33 (36.7%) patients with a duration of ≤3 years were positive for both IA-2A and ZnT8A, respectively. The prevalence of both IA-2A and ZnT8A saw a significant decrease relating to disease duration, and <20% of patients with a duration of ≥16 years were positive for these autoantibodies. A total of 65 (72.2%) patients with a duration of ≤3 years were positive for GADA. Although a significant decline in GADA positivity was also observed, approximately 50% of patients with a duration of ≥16 years remained positive. Our combined analysis showed that the prevalence of patients positive for one or more of the autoantibodies also saw a significant decrease from 80% to 62% ($P = 0.0064$; Table 1).

Interaction of duration of diabetes and age of onset on anti-islet autoantibody positivity

Table 2 shows the results of four separate multivariate logistic regression analyses with dependent variables IA-2A, ZnT8A, GADA or ≥1Ab. In patients whose age of onset was ≤10 years, positivity of anti-islet autoantibodies decreased significantly for patients with a duration of diabetes ≥16 years compared with

Table 1 | Univariate analysis for the prevalence of autoantibodies to glutamic acid decarboxylase, insulinoma-associated antigen-2 and zinc transporter 8

	n	GADA			IA-2A			ZnT8A			≥1Ab		
		%Pos	OR	95%CI	%Pos	OR	95%CI	%Pos	OR	95%CI	%Pos	OR	95%CI
Age at onset													
0–10 years	85	48.2	1	–	41.2	1	–	34.1	1	–	69.1	1	–
11–30 years	89	65.2	**2.01***	1.09–3.69	27.0	**0.53***	0.28–1.00	21.3	0.52	0.27–1.03	75.3	1.34	0.69–2.62
≥31 years	95	74.7	**3.18****	1.69–5.95	28.4	0.57	0.31–1.06	20.0	**0.48***	0.25–0.95	80.0	1.76	0.89–3.49
Duration of diabetes													
0–3 years	90	72.2	1	–	41.1	1	–	36.7	1	–	80.0	1	–
4–15 years	92	67.4	0.80	0.42–1.50	37.0	0.84	0.46–1.52	21.7	**0.48***	0.25–0.92	82.6	1.19	0.56–2.51
≥16 years	87	49.4	**0.38****	0.20–0.70	17.2	**0.30****	0.15–0.60	16.1	**0.33****	0.16–0.68	62.1	**0.41***	0.21–0.80

%Pos, percentage of autoantibody-positive patients; CI, coefficient interval; GADA, glutamic acid decarboxylase autoantibodies; IA-2A, insulinoma-associated antigen-2 autoantibodies; OR, odds ratio; ZnT8A, zinc transporter 8 autoantibodies. Bold values indicate statistically significant difference. *$P < 0.05$. **$P < 0.005$.

Table 2 | Multivariate logistic regression analysis for the prevalence of autoantibodies to glutamic acid decarboxylase, insulinoma-associated antigen-2 and zinc transporter 8

Age at onset	Duration of diabetes	*n*	GADA			IA-2A			ZnT8A			≥1Ab		
			%Pos	OR	95%CI	%Pos	OR	95%CI	%Pos	OR	95%CI	%Pos	OR	95%CI
0–10 years	0–3 years	32	62.5	1	–	46.9	1	–	50.0	1	–	78.1	1	–
	4–15 years	25	48.0	0.55	0.19–1.60	60.0	1.70	0.59–4.90	28.0	0.38	0.13–1.19	84.0	1.47	0.38–5.72
	≥16 years	28	32.1	**0.28***	0.10–0.83	17.9	**0.25***	0.08–0.81	21.4	**0.27***	0.09–0.85	42.9	**0.24***	0.08–0.74
11–30 years	0–3 years	27	77.8	1	–	29.6	1	–	33.3	1	–	81.5	1	–
	4–15 years	27	66.7	0.57	0.17–1.92	29.6	1	0.31–3.22	22.2	0.57	0.17–1.92	77.8	0.80	0.21–3.01
	≥16 years	35	54.3	0.34	0.11–1.05	22.9	0.70	0.23–2.21	11.4	**0.26***	0.07–0.96	68.6	0.50	0.15–1.66
≥31 years	0–3 years	31	77.4	1	–	45.2	1	–	25.8	1	–	80.6	1	–
	4–15 years	40	80.0	1.17	0.37–3.66	27.5	0.46	0.17–1.24	17.5	0.61	0.19–1.92	85.0	1.36	0.39–4.72
	≥16 years	24	62.5	0.49	0.15–1.58	8.3	**0.11***	0.02–0.55	16.7	0.58	0.15–2.20	70.8	0.58	0.17–2.04

*$P < 0.05$. %Pos, percentage of autoantibody-positive participants; CI, coefficient interval; GADA, glutamic acid decarboxylase autoantibodies; IA-2A, insulinoma-associated antigen-2 autoantibodies; OR, odds ratio; ZnT8A, zinc transporter 8 autoantibodies. Bold values indicate statistically significant difference.

those with a duration ≤3 years (odds ratio [OR] 0.25–0.28, $P < 0.05$). However, in patients from the middle tertile (11–30 years), only ZnT8A positivity saw a decrease in frequency for patients with a duration of diabetes ≥16 years when compared with those with a duration ≤3 years (OR 2.26, $P < 0.05$). Furthermore, the interaction was significant only for IA-2A in those with an onset age of ≥31 years (OR 0.11, $P < 0.05$). ≥1Ab was an admixture of the results for GADA, IA-2A and ZnT8A. For patients with onset of diabetes at age ≤10 years, positivity also decreased significantly in patients with duration of diabetes of ≥16 years compared with those with a duration ≤3 years (OR 0.24, 95% confidence interval 0.08–0.74, $P < 0.05$). However, the interaction was not significant for patients from the other two age tertiles (11–30 years and ≥31 years).

Others have reported that slowly progressive type 1 diabetic patients[14,15] or females[7,16] are more likely to be GADA-positive than acute-onset type 1 diabetes or males, respectively. Univariate analysis showed that GADA positivity is more likely in those with slowly progressive type 1 diabetes (OR 2.24, 95% confidence interval 1.14–4.41, $P = 0.020$), but not females (Table S2). However, multivariate analysis, including onset age and duration of disease, sex, and subtype of type 1 diabetes, did not affect the significance of the interaction of onset and duration on GADA, IA-2A, and ZnT8A (data not shown).

DISCUSSION

In the present study, we showed that: (i) both IA-2A and ZnT8A were frequent in younger patients, whereas GADA were more common in older patients; (ii) the prevalence of IA-2A, ZnT8A and GADA declined according to the disease duration; and (iii) significant interaction was observed between the age of onset and duration of diabetes in patients with an onset age of ≤10 years regarding IA-2A, ZnT8A and GADA.

The present study's principal objective was to learn how the prevalence of anti-islet autoantibody changes after disease onset, and how understanding its relationship to the age of onset might help to avoid the misclassification of diabetes. As anti-

islet autoantibody measurement can offer utility in backing up clinical acumen in determining how to best manage patients with diabetes, a basic understanding of each autoantibody's dynamics is vital in general practice. The data we have presented suggest IA-2A, ZnT8A and GADA's rate of disappearance is faster in patients with an age of onset ≤10 years and relates to the disease duration. Therefore, to effectively discriminate between type 1 diabetes and other monogenic diabetes, such as Wolfram syndrome and maturity-onset diabetes of the young[17–19], cystic fibrosis[20] or ketosis-prone diabetes[21], we assert the importance of early examination of the anti-islet autoantibodies in this patient group.

The diagnostic sensitivity of the anti-islet autoantibody tests we investigated tended to vary according to age at diabetes onset, with IA-2A and ZnT8A being higher in children, and GADA being higher in adults, as reported in previous studies[6–8]. Furthermore, as previously stated, it has been reported that GADA's persistent presence is associated with thyroid autoimmunity in patients with type 1 diabetes[9,22] and the chromosome, 3q28, in a region of strong linkage disequilibrium in the first intron of the gene, *LPP*[16], which was originally identified as a susceptibility locus for celiac disease[23] and autoimmune thyroid disease[24]. Furthermore, in longitudinal studies of genetically at-risk children followed from birth, it has been reported that the presence of multiple anti-islet autoantibodies greatly increases the probability for type 1 diabetes[25], and IA-2A and ZnT8A are shown to appear later, in general, during the subclinical disease process and to herald more rapid progression to type 1 diabetes than GADA[26,27]. These data imply that anti-GAD response might be a sign of general autoimmunity, whereas IA-2A and ZnT8A might be a surrogate indicator of residual β-cell mass[28–30], although there is a study showing that the prevalence of IA-2A and ZnT8A at and after type 1 diabetes onset did not strongly correlate with endogenous insulin secretion[31]. To avoid autoimmune thyroid disease convoluting the results of the present study, we excluded the type 1 diabetic patients with autoimmune thyroid disease who were persistently positive for GADA[9]. Because of this exclusion, we suggest that the GADA persistence in patients aged ≥11 years observed in the present study is unrelated to the presence of autoimmune thyroid disease.

Although the age-dependent decline of the autoantibody positivity is similar between IA-2A and ZnT8A, the interaction between the onset age and duration of diabetes was different between the two autoantibodies. The interaction was significant only for ZnT8A in patients diagnosed in the middle tertile (11–30 years), and only for IA-2A in those with late-onset diabetes (aged ≥31 years). These results show that the intensity of the autoimmune response to IA-2 and ZnT8A can vary according to age, even though both autoantigens are transmembrane proteins located within the insulin secretory granules and the half-life of both autoantibodies is correlated[30]. In a study on a population of white European origin, Tridgell *et al.*[32] reported that the interaction between the onset of disease and duration of diabetes was significant for GADA, but not IA-2A, in type 1 diabetic patients. In contrast with the results from the present study, they showed that the prevalence of GADA decreased rapidly after diagnosis in patients aged <14 years, whereas the decline in IA-2A positivity was similar regardless of the age of onset. Furthermore, the age-dependent associations between anti-islet autoantibody positivity and genetic factors, such as human leukocyte antigen[7], and the relationship between ethnicity and autoantibody status were also reported[8]. Previous studies showed that the HLA-DR3-DQ2 haplotype, which is absent or very rare in the Japanese population[33], was positively associated with GADA[7,16]. In a cross-study comparison, the results led to the speculation that ethnic differences might be one of the reasons for the discrepancies between the two studies, as the genetic background of Japanese type 1 diabetic patients differs from that of white patients[5]. However, a longitudinal study, including genetic factors, is required to confirm whether the interaction between the age- and duration-dependent effects on anti-islet autoantibody positivity in the Japanese population is distinct from that of the white population.

There were several limitations to this study, to begin with, the number of participants was relatively small, which might raise some imbalances in boundaries of age at onset and duration of diabetes. Therefore, further investigation using a larger cohort is required to confirm the present results. Additionally, we did not have genetic or metabolic data, and so did not examine the association between the presence of autoantibodies and genetic background or loss of β-cell function.

In summary, the present study showed that the disappearance of anti-islet autoantibodies is more rapid in patients aged ≤10 years at onset, and that even though both proteins are localized in the insulin granule membrane, humoral autoimmunity to IA-2 and ZnT8 differs according to the age of onset. Therefore, we conclude that the age of onset and duration of diabetes both have a critical impact on autoantibody status, and should be considered when interpreting a result for patients with diabetes. Although the exact mechanisms by which these anti-islet autoantibodies generate an age-dependent effect are still unknown, the data presented in the present study should provide a compelling context for clinicians and investigators using autoantibody measurement to classify individuals with diabetes.

ACKNOWLEDGMENTS

This research has not received any specific grants from funding agencies in the public, commercial or not-for-profit sectors.

DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Clinical characteristics.
Table S2 | Prevalence of anti-islet autoantibodies and their association with female sex and slowly progressive type 1 diabetes.