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Significance of IA-2 antibody in Japanese type 1 diabetes: its association with GAD antibody

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Abstract

To investigate the presence and level of serum antibodies to IA-2 (IA-2A) in Japanese patients with adult type 1 diabetes in order to clarify its association with glutamic acid decarboxylase (GAD) antibody. Serum samples were obtained from 101 Japanese patients with type 1 diabetes, including 37 patients with slowly progressive form of type 1 diabetes. Serum levels of IA-2A and GADA were determined by radioimmunoassay. The study had a cross-sectional design. IA-2A and GADA were detected in 37 and 59% of these patients, respectively. Of the 37 slowly progressive form of patients, IA-2A and GADA were present in 49 and 86%, respectively (NS). GADA levels were significantly higher ($P < 0.05$) in IA-2A positive than in IA-2A negative patients in slowly progressive form, but IA-2A levels did not differ significantly between GADA positive and GADA negative patients. Measuring IA-2A in combination with GADA is useful for the diagnosis and prognosis of type 1 diabetes in Japanese.

Keywords: Islet cell autoantibodies; IA-2A; GADA; Type 1 diabetes; Autoimmune thyroid disease

1. Introduction

Type 1 diabetes is a heterogeneous disorder, resulting in most cases from autoimmune mediated destruction of pancreatic beta cells, which leads to an absolute deficiency of insulin production. The rate of destruction is quite variable, being rapid in some individuals and slow in others [1,2]. The slowly evolving form of type 1 diabetes is known as slowly progressive insulin-dependent diabetes mellitus (SPIDDM) [1] or latent autoimmune diabetes in adults (LADA) [2].

Prior to the onset of the disease, autoantibodies that recognize islet antigens may appear, and these autoantibodies may be used to predict those who will develop type 1 diabetes [3–6]. Islet cell antibody (ICA) has been utilized to diagnose type 1 diabetes because this antibody circulates in most patients before and at the onset of the disease [3]. At least two

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target antigens have been identified. The first is glutamic acid decarboxylase (GAD), the enzyme that catalyzes the decarboxylation of glutamic acid to γ-aminobutyric acid [4], and the second is the protein tyrosine phosphatase-related molecule ICA-512 [5] or IA-2 [6]. Antibodies to both enzymes are often present in the sera of type 1 diabetics before the onset of the disease [7–10]. Autoantibodies to glutamic acid decarboxylase (GADA) are frequently detected in slow onset form of type 1 diabetes and indicate the development of insulin dependency [11,12]. In contrast, autoantibodies to tyrosine phosphatase ICA512/IA-2 (IA-2A) are found in some sera that are negative for GADA at the onset of diabetes [9,10], suggesting that these two major antibodies may be diagnostic for different subsets of type 1 diabetes. However, the presence of IA-2A and GADA, and their clinical usefulness in Japanese type 1 diabetic patients have not been fully determined. We investigated the significance and association of serum antibodies to IA-2A and GADA in Japanese patients with type 1 diabetes.

2. Materials and methods

2.1. Human subjects

We studied 101 Japanese patients with type 1 diabetes, who fulfilled the classification of the American Diabetes Association [13] as described previously [14]. Included were 47 men and 54 women, aged 41.3 ± 15.3 (mean ± S.D.) years of age (range 14.0–89.0 years), who had diabetes for 10.4 ± 9.6 years. Informed consent was obtained from each subject studied. Diagnosis of type 1 diabetes was determined clinically and confirmed by a basal serum C-peptide level of less than 1.0 ng/ml and/or urinary excretion of C-peptide less than 20 μg/day.

Patients were classified into two groups according to their time course of insulin dependency [1,15,16]. Those with acute onset type 1 diabetes ($n = 64$) had a history of ketonuria or ketoacidosis and/or required insulin therapy at the time of diagnosis or within 6 months of diagnosis, while those with a slowly progressive form ($n = 37$) did not require insulin for more than 6 months after diagnosis (Tables 2 and 3). Serum was obtained from each patient and kept at –80 °C until assayed for autoantibodies.

2.2. Assay for GAD antibody

Serum GADA levels were determined using a commercially available radio-immunoassay kit (RSR Ltd., Cardiff, UK) and human recombinant GAD65 [17] on the basis of the first proficiency test of the Diabetes Autoantibody Standardization Programs (DASP) [18]. Briefly, 50 μl of the tracer reagent ^{125}I-labeled recombinant human GAD65, were incubated with 20 μl of undiluted test serum at room temperature. Unbound labeled GAD was separated by adding solid phase protein A, and the specific radioactivity in the precipitates was counted. The GADA concentration in each sample was determined by comparison with a calibration curve plotted from levels of standard serum. The intra- and interassay coefficients of variation (CVs) were 8.0 and 8.6%, respectively. Serum samples were considered positive if the GADA concentration exceeded 1.3 U/ml, which was 3 S.D. above the control mean [19]. All samples were tested in duplicate, including positive and negative control sera.

2.3. Assay for IA-2 antibody

Serum IA-2A levels were determined using an ICA512 radio-immunoassay kit, (RSR Ltd.) and human recombinant ICA512 [20] on the basis of the DASP [18]. Briefly, 50 μl of ^{125}I-labeled ICA512 solution were incubated with 20 μl of undiluted test serum at room temperature. Solid phase protein A was added to remove unbound labeled ICA512, and the radioactivity in the precipitates was counted. The ICA512 level in each sample was determined by comparison with a calibration curve plotted from levels of standard serum. The intra- and interassay CVs were 2.6 and 1.3%, respectively. Serum samples were considered positive if the IA-2A concentration exceeded 0.4 U/ml, which was 3 S.D. above the control mean. All samples were tested in duplicate, including positive and negative control sera.

2.4. Assay for thyroid autoantibodies

Serum concentrations of antibodies to thyroglobulin (TgAb) and thyroid peroxidase (TPOAb) were measured using a commercially available radioimmunoassay kit (RSR Ltd.) [21]. The cutoffs were >0.3 U/m for TgAb and TPOAb [19].