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ORIGINAL ARTICLE

Autoantibodies against aminoacyl-tRNA synthetase: novel diagnostic marker for type 1 diabetes mellitus

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Abstract

Objectives: To investigate whether or not antiaminoacyl-tRNA synthetase (aaRS) autoantibodies could be detected in patients with type 1 diabetes mellitus (DM) and be used as a diagnostic marker for type 1 DM, autoantibodies against aaRSs were measured in the plasma of normal subjects, patients with type 1 DM and patients with type 2 DM.

Methods: An enzyme-linked immunosorbent assay was performed to detect anti-aaRS autoantibodies in the plasma of normal subjects, and patients with type 1 DM, and patients with type 2 DM.

Results: From the 65 (normal), 58 (type 1 DM) and 57 (type 2 DM) subjects, anti-aaRS autoantibodies were found in 37.9% of patients with type 1 DM compared with 1.54% of the non-diabetic controls, and 5.26% of the patients with type 2 DM (p <0.0001). In addition, anti-aaRS autoantibodies were identified in 30% of patients with type 1 DM without classical type 1 DM autoantibodies.

Conclusion: Anti-aaRS autoantibodies were identified in 37.9% of patients with type 1 DM. The results of this study demonstrate for the first time that autoantibodies against aaRSs are specifically associated with type 1 DM.

Keywords: *Pancreas; autoantibody; antisynthetase syndrome; autoimmune disease; ELISA*

Introduction

Aminoacyl-tRNA synthetases (aaRSs) are composed of 20 different enzymes that catalyse the ligation of a specific amino acid to its cognate tRNA, thereby resulting in the precise translation of genetic information (Kisselev et al. 1998, Park et al. 2005a). The expression of aaRSs has been reported to be specifically enriched in some tissues such as the heart, muscles and pancreas (Antonellis et al. 2003, Scheinker et al. 1979, Favorova et al. 1989). Recently, many studies have investigated the presence of aaRSs in tissues. The findings of such studies have shown that aaRSs plays an important role in the regulation of a variety of cellular and physiological processes, in addition to protein synthesis. For example, aaRS regulates the packaging of the human immunodeficiency virus (HIV) virion and activates the immune system by activation of transcription factors in the cytoplasm, and it inhibits Fas-mediated apoptosis (Kaminska et al. 2007, Kovaleski et al. 2006, Ko et al. 2001). In addition, aaRSs are secreted from cells, and regulate angiogenesis and the cytokines associated with inflammation (Park et al. 2005b, Tzima et al. 2005, Wakasugi et al. 2002).

Recently, it has been reported that the abnormal expression of aaRS is directly or indirectly associated with a variety of diseases. The mutation of some cytoplasmic aaRSs

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induces neuronal defects resulting in the development of Charcot–Marie–Tooth, a group of disorders characterized by axonal demyelination (type 1) or decreased amplitude of evoked motor and sensory responses (type 2) (Dyck & Lambert 1968, Antonellis et al. 2003, Jordanova et al. 2006, Nangle et al. 2007). In some cases, mutations of cytoplasmic aaRSs cause abnormalities in the editing activity involved in the clearing of mischarged aminoacyl-tRNA. This results in the incorporation of mischarged amino acids into proteins and the accumulation of proteins with an abnormal structure. The accumulation of misfolded proteins triggers cellular toxicity, eventually leading to apoptosis (Nangle et al. 2006, Lee et al. 2006). In addition, a specific mutation of mitochondrial aspartyl-tRNA synthetase (mDRS) has been associated with leukoencephalopathy, which has brain stem and spinal cord involvement and lactate elevation (Scheper et al. 2007). The individuals develop progressive cerebellar ataxia, spasticity and dorsal column dysfunction. The aaRS interacting multifunctional protein 1 (AIMP1) is enriched in the pancreatic alpha cells. AIMP1 is secreted from the pancreatic alpha cells under fasting conditions and induces the secretion of glucagon (Park et al. 2006). In addition, AIMP1 induces glycogenolysis to increase blood glucose level. AIMP1 knock-out mice shows defects in blood metabolites (Park et al. 2006).

Furthermore, aaRSs have been reported to be targeted as autoantigens (Mathews & Bernstein 1983). Autoantigenic aaRSs induce migration of CD4+ and CD8+ T cells, monocytes and immature dendritic cells (Howard et al. 2002). The uptake and surface presentation of autoantigenic aaRSs by antigen-presenting cells, mediate immune responses against the self-antigens. Autoantibodies against aaRS have been identified in patients with autoimmune diseases suffering from inflammatory myositis (Yoshifuji et al. 2006, Adam et al. 2007, Hirakata et al. 1996). About 30% of the patients with autoimmune diseases have anti-aaRS autoantibodies (Mathews & Bernstein 1983). Patients with anti-aaRS autoantibodies have the antisynthetase syndrome, which includes such disorders as idiopathic inflammatory myopathies, interstitial lung diseases, arthritis, Raynaud's phenomenon and fevers, which are systemic diseases characterized by chronic muscle inflammation. The investigation of patients with various autoimmune diseases might be useful for understanding the correlation of anti-aaRS autoantibodies with their specific diseases. At present, there is no explanation as to why aaRSs are targeted as autoantigens. However, it is clear that anti-aaRS autoantibodies are associated with specific autoimmune diseases as described above (Koreeda et al. 2010).

Type 1 diabetes mellitus (DM) is an autoimmune disease caused by the destruction of insulin-producing pancreatic beta cells by autoreactive T cells recognizing autoantigens (Mauricio & de Leiva 2001, Sakumai et al. 2007, Kantarova & Buc 2007). Humoral and cellular immune responses have been associated with type 1 DM. Autoantibodies against a variety of islet-cell antigens (ICA) are present in the sera of patients with type 1 DM. Autoantibodies against glutamic acid decarboxylase 65 (GAD65), insulin and protein tyrosine phosphatase-related islet antigen 2 (IA-2) are being used to diagnose type 1 DM (Bilbao et al. 2000, Taplin & Barker 2008, Knip & Siljander 2008). The sensitivity of GAD65, insulin and IA-2 is about 60–80%, 40–60% and 30–70%, respectively (Taplin & Barker 2008). The combination of these three major autoantibodies can predict 80% or more of patients with type 1 DM or patients at risk for the development of type 1 DM. To date, many approaches have been tried to block the exacerbation of type 1 DM. For instance, immunotherapy using autoantigens in patients with diabetes has been shown to enhance beta cell regeneration, and in some cases to induce remission of diabetes (Nishio et al. 2006). Therefore, novel autoantigens may be useful for antigen-based immunotherapy as well as potential diagnostic markers for type 1 DM, such as zinc transporter 8 (ZnT8), which has recently been identified as a major autoantigen associated with type 1 DM (Wenzlau et al. 2007).

Autoantibodies against aaRS are found in patients with autoimmune disease and some aaRSs are specifically enriched in the pancreas (Antonellis et al. 2003, Kron et al. 2005). The goal of this study was to investigate the potential of aaRSs as novel autoantigens in patients with type 1 DM. Herein, the findings that some aaRSs are mainly enriched in pancreatic beta cells compared with acinar cells is reported, and the presence of autoantibodies against aaRSs were present in patients with type 1 DM. The results suggest that aaRSs might be novel autoantigens associated with type 1 DM, and these autoantibodies might be potential markers for the diagnosis of type 1 DM.

Materials and methods

Human subjects

Fifty-eight patients with type 1 DM, 65 age-matched non-diabetic control subjects, and 57 patients with type 2 DM were studied. All patients with type 1 and type 2 diabetes fulfilled the classification criteria of the American Diabetes Association. Prior to patient participation, formal approval of the study was obtained from the patients. The clinical characteristics of the subjects are summarized in Table 1.

Protein expression and purification

The sequences encoding glycyl-tRNA synthetase (GRS), asparaginyl-tRNA synthetase (NRS), alanyl-tRNA synthetase (ARS) and tryptopohanyl-tRNA synthetase (WRS) were

Table 1. Clinical characteristics of the study subjects.

	NC ($n=65$)	TIDM ($n=58$)	T2DM ($n=57$)
Age (years)	35.1 ± 7.5	35.4 ± 13.1	57.1 ± 7.7
Sex (M:F)	31:34	25:33	29:28
Duration of diabetes (years)	NA	6.6 ± 7.5	7.2 ± 7.2
BMI (kg m^{-2})	22.9 ± 3.1	20.9 ± 2.8	23.6 ± 3.1
Fasting C-peptide (ng ml^{-1})	NA	0.52 ± 0.52	NA

Data are means ± SD. NC, non-diabetic control; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; BMI, body mass index; NA, not applicable.

subcloned into pET28a (Novagen, Darmstadt, Germany) and overexpressed in *Escherichia coli* BL21 (DE3) by induction with 0.5 mM IPTG at 23°C for 12 h. The cells were harvested, lysed by sonication in buffer A (50 mM Tris–HCl, 50 mM NaCl, 0.5 mM EDTA, 2 mM 2-mercaptoethanol, pH 7.8) and centrifuged (22 000 *g*). The supernatant was precipitated with ammonium sulfate with gentle swirling. The fraction between 25% and 50% was dialysed with Buffer B (50 mM Tris–HCl, 100 mM NaCl, 0.5 mM EDTA, 2 mM 2-mercaptoethanol, 15% glycerol, pH 7.6) and loaded onto a SP-sepharose column (Bio-Rad, Hercules, CA, USA). The proteins were eluted with a 0.7 M NaCl gradient and again with a heparin column (Bio-Rad). The proteins were loaded onto a Ni^{++}-column (Invitrogen, Carlsbad, CA, USA) and eluted with 250 mM imidazole. Then the proteins were dialysed against 1x PBS and stored at –70°C. The purified proteins had a purity over 95% at Coomassie staining.

ELISA

GAD65, IA-2 and ICA enzyme-linked immunosorbent assay (ELISA) kits were purchased from Biomerica and an ELISA was performed following the manufacturer's protocol. For the analysis of aaRS autoantibodies from human plasma, each well was coated with 2 ng ml^{-1} of aaRSs for 12 h at 4°C and blocked with PBS containing 2.5% BSA for 1 h at room temperature (RT). Addition of 1/100 diluted human plasma to each well was carried out and the samples were incubated for 1 h at RT. After washing the plate with PBS containing 0.1% Tween 20, 1/10 000 of diluted HRP-conjugated antihuman antibody was added to each well and incubated for 1 h at RT. After washing the plate with washing buffer, we added substrate and developed it for 20 min at RT protected from the light. The reaction was stopped by the addition of 1 N HCl and read at 450 nm.

Western blot analysis using patient serum

Ten nanograms of each purified aaRSs sample was loaded onto 10% SDS-PAGE out and then transferred onto a PVDF membrane (0.4 μm; Millipore, Billerica, MA, USA). The membranes were blocked with TBST containing 5% BSA for 1 h at RT. Each human plasma sample was diluted to 1/100 with TBST containing 1% BSA and the membranes were incubated for 1 h at RT. After washing the membranes, 1/10000 diluted HRP-conjugated antihuman antibody was added to the membranes and developed.

Immunofluorescence staining

The dewaxed sections (6 μm) of the pancreas were heated with 0.1 mM citrate solution (pH 6.0). The sections were incubated with 0.5% Triton X 100/PBS solution for 15 min and washed with PBS three times. To block non-specific binding, normal goat serum was diluted in PBS containing 0.5% Triton X-100, 0.3% BSA, and the slides were incubated for 60 min at RT. Primary antibodies were loaded onto the slides and incubated for overnight at 4°C in a humidified chamber. After washing the slides with 1x PBST, sections were incubated with Alexa Fluor 594- or Alexa Fluor 488-conjugated secondary antibodies (Invitrogen) to visualize aaRSs or insulin, respectively. The cell nuclei were counterstained with 4′–6′ diamidino-2-phenylindole (DAPI) (Invitrogen).

Data analysis

Positive signals for GAD65, IA-2 and ICA ELISA using human plasma were determined according to the manufacturer's instructions. The analysis of the controls, patients with type 1 DM and patients with type 2 DM, had an intra-assay of coefficient of variation (CV) ranging from 11 to 18% and an interassay CV of 17–24%. The cut-off values were 2.5 of the mean for the non-diabetic subjects compared with the Western blot analysis at the 98% cut-off. The assay had 47–62% sensitivity and 87–100% specificity. Statistical differences among the groups were determined by the χ^2 test, and the differences among the groups were considered significant when $p < 0.05$.

Results

Localization of aaRSs within the pancreas

Recent reports have shown that expression of some aaRSs can be found specifically in the pancreas (Antonellis et al. 2003, Kron et al. 2005). However, suborganellar localization of aaRSs within the pancreas has not been confirmed. Because the pancreas is composed of two different systems – the endocrine and exocrine systems – we first investigated the expression profile of aaRSs in the human pancreas. We carried out indirect immunofluorescence staining of the pancreas using specific antibodies against ARS, GRS, NRS and WRS. The results showed that ARS and GRS were mainly and specifically enriched in the beta cells of the islets of Langerhans (Figure 1C and D). However, NRS and WRS were evenly distributed

among the acinar cells and islets of Langerhans in the pancreas (Figure 1E and F).

Autoantibody analysis using human plasma

We purified ARS, GRS, NRS and WRS as described in the Methods section. The protein purity was at least 95% by SDS-PAGE (data not shown). ELISA using purified aaRSs showed that anti-ARS autoantibodies were found in insulin-treated patients with type 1 DM, patients with type 2 DM, and in the non-diabetic control subjects. To confirm the specificity and to rule out possible false-positive results, the ELISA results were validated by competition assays of all samples (normal, 65 subjects; type 1 DM, 58 subjects; type 2 DM, 57 subjects) using their specific antigens. For the competition assay, the specific soluble antigen was pre-incubated with plasma, and then the mixture was loaded into antigen-coated wells. Competition assay showed that the soluble antigen efficiently blocked the binding of the plasma autoantibodies to the coated antigen in a dose-dependent manner (Figure 2A). However, the BSA, an irrelevant antigen, did not inhibit the binding

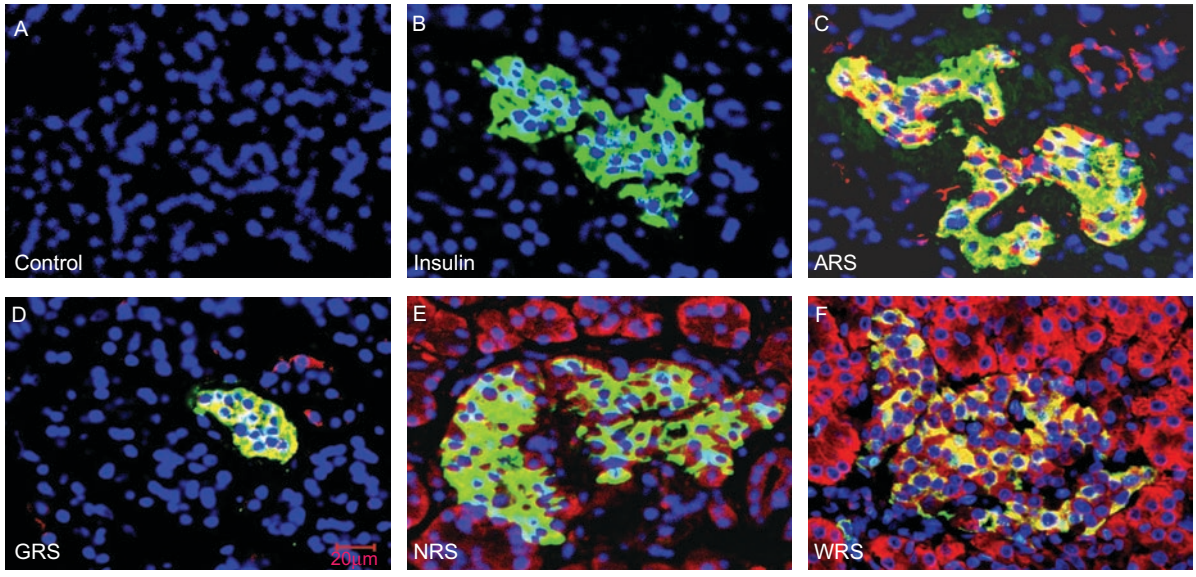

Figure 1. Localization of aminoacyl-tRNA synthetases (aaRSs) in the human pancreas. Sections of 6 μm of formalin-fixed paraffin tissue were used for immunofluorescence staining. Insulin (green) was stained with FITC-conjugated antimouse antibody (B), and alanyl-tRNA synthetase (ARS) (C), glycyl-tRNA synthetase (GRS) (D), asparaginyl-tRNA synthetase (NRS) (E) and tryptopohanyl-tRNA synthetase (WRS) (F) were stained with Alexa Fluor 555-conjugated antirabbit antibodies (red). The control was stained with rabbit mock IgG (A). The colocalized signals are shown in yellow. The nuclei were counterstained with diamidino-2-phenylindole (blue). Scale bar, 20 μm.

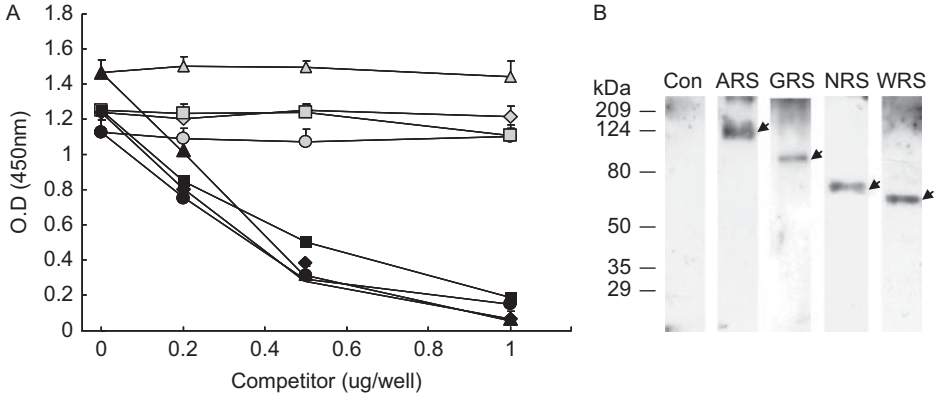

Figure 2. Validation assay using soluble antigens. (A) For the competition assay using soluble aminoacyl-tRNA synthetases (aaRS) antigens, each aaRS (black oval, alanyl-tRNA synthetase, ARS; black diamond, tryptopohanyl-tRNA synthetase, WRS; black triangle, glycyl-tRNA synthetase, GRS; black square, asparaginyl-tRNA synthetase, NRS) or BSA (grey circle, ARS; grey diamond, WRS; grey triangle, GRS; grey square, NRS) was preincubated with anti-aaRS autoantibody-positive human plasma, and loaded into each antigen-coated well, and then analysed. (B) Western blot analysis of ARS, GRS, NRS and WRS using plasma from patients with type I diabetes mellitus (DM). Each 10 ng of purified ARS, GRS, NRS and WRS was loaded on to 10% reducing SDS-PAGE, and blotted with the plasma of patients with type I DM as described in the Methods section. The arrow indicates the expected size of each aaRS.

of the plasma autoantibodies to antigen. Validation using the antigen competition assay successfully excluded the false-positive subjects. In addition, whether or not the plasma of type 1 DM subjects positive for the anti-aaRS autoantibody could specifically recognize each aaRS was investigated. Immunoblot analysis using purified ARS, GRS, NRS and WRS showed that the human plasma specifically recognized each of the aaRSs. However, the plasma of the non-diabetic control subjects did not recognize the aaRSs (Figure 2B). In the non-diabetic and type 1 DM subjects, two out of three subjects and three out of 24 subjects had false-positive findings. Anti-aaRS autoantibodies were significantly found in 36.2% of type 1 DM subjects compared with 1.54% of the non-diabetic control subjects and 5.26% of the type 2 DM subjects (Table 2). These differences were statistically significant.

We further compared the frequency of autoantibodies from subjects of each group after validation. The validation assay clearly showed that anti-ARS autoantibodies were found significantly in type 1 DM subjects compared with type 2 DM subjects (24.1% vs 5.3%, p <0.01) and the non-diabetic control subjects (24.1% vs. 1.5%, p <0.01) (Figure 3A). Anti-GRS autoantibodies were found in 1.7% of the subjects with type 2 DM, in 8.6 % of subjects with type 1 DM, and 1.5% of the non-diabetic control subjects (Figure 3B). In addition, anti-NRS autoantibodies were found significantly in type 1 DM subjects compared with the non-diabetic control subjects (13.8% vs 1.5%, p <0.05) and the subjects with type 2 DM (13.8% vs 3.5%) (Figure 3C). Anti-WRS autoantibodies were also found significantly in subjects with type 1 DM compared with the non-diabetic control subjects (12.1% vs 1.5%, p <0.05) and type 2 DM subjects (12.1% vs 3.5%) (Figure 3D). Moreover, the anti-GRS autoantibody population completely overlapped with the anti-NRS autoantibody population (data not shown). The unique populations of ARS, NRS and WRS showed levels of 12.1%, 5.2% and 6.9%, respectively. ARS and NRS was a double positive population with 6.9% levels. ARS and WRS was a double population with 3.4% levels. However, there was no double positive population for NRS and WRS (Figure 3E). Taken together, these results suggest that anti-aaRS autoantibodies might be useful markers for type 1 DM.

Table 2. Validation of autoantibodies against antiaminoacyl-tRNA synthetases.

	No. of positives	No. of false positives	No. of real positives	Real positives (%)	p-Value
Type 1 DM (n=58)	24	3	21	36.2	p <0.001 (vs normal or type 2)
Type 2 DM (n=57)	3	0	3	5.26	NS
Normal (n=65)	3	2	1	1.54	NA

DM, diabetes mellitus; NS, not significant; NA, not applicable.

Comparative analysis of aaRSs with GAD, IA-2 and ICA

To compare the sensitivity of aaRSs detection with GAD65, IA-2 and ICA in the plasma of patients with

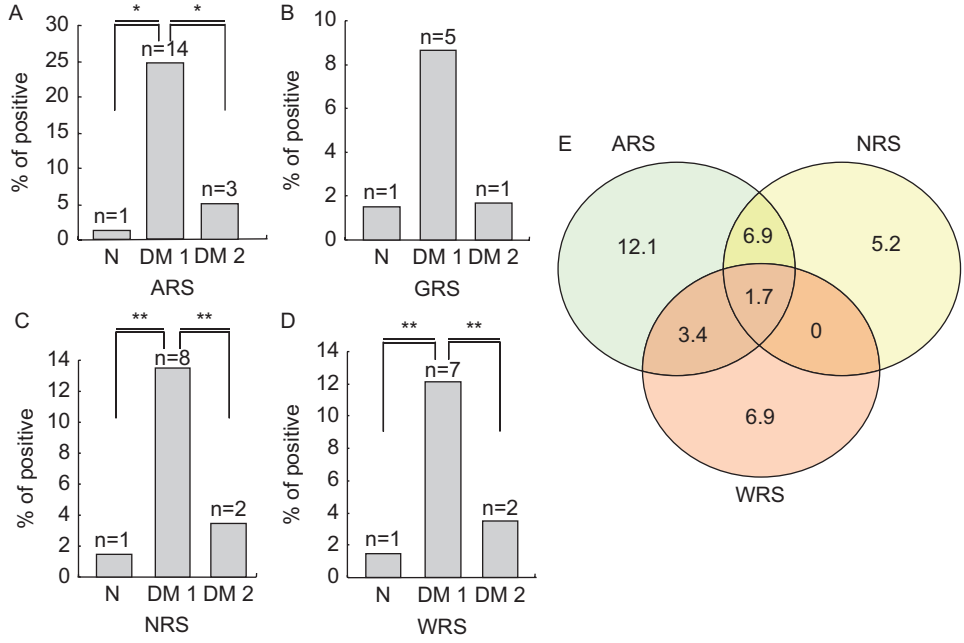

Figure 3. Summary of anti-aminoacyl-tRNA synthetase (aaRS) autoantibody-positive populations. The anti-alanyl-tRNA synthetase (ARS) (A), glycyl-tRNA synthetase (GRS) (B), asparaginyl-tRNA synthetase (NRS) (C), tryptopohanyl-tRNA synthetase (WRS) (D) autoantibodies after validation are summarized. Each specific position and the rate of overlapping of the autoantibodies are described in the diagram. *p <0.01; **p <0.05.

type I DM (used in this study), we carried out an ELISA using the anti-GAD65, -IA2 and -ICA autoantibody detection kit. For the type 1 DM plasma, anti-GAD65, -IA2 and -ICA autoantibodies were detected at in 62.1%, 24.1%, and 22.4%, respectively. IA-2-positive populations completely overlapped with GAD and ICA. In addition, the IA-2-positive populations did not overlap with aaRS-positive populations (data not shown), indicating no specificity for IA-2 (Figure 4A). Only 19% of patients were double positive for both ICA and GAD65 (Figure 4A).

Next, the specificity of each aaRS autoantibody was analysed for the diagnosis of type 1 DM in conjunction with GAD65, IA-2 and ICA autoantibodies. Among the anti-ARS autoantibody-positive population, 8.6% were double positive for anti-GAD65 and anti-ICA autoantibodies, and 10.4% were positive only for the anti-GAD65 autoantibody. There were 5.2% out of 24.1% of the anti-ARS autoantibody-positive population who were positive only for anti-ARS autoantibodies (Figure 4B). In the case of the anti-GRS autoantibody-positive population, no-one was uniquely positive to the anti-GRS autoantibody (Figure 4C). In the analysis of the anti-NRS autoantibody-positive population, 6.9% were double positive for anti-GAD65 and anti-ICA autoantibodies, and 3.5% were positive for only the anti-GAD65 autoantibodies. There were 3.5% out of 13.8% that had a unique response to the anti-NRS autoantibody (Figure 4D). In the case of anti-WRS autoantibodies, 5.2% of the anti-WRS autoantibody-positive population was positive for both anti-GAD65 and anti-ICA autoantibodies. Of the anti-WRS autoantibody-positive population, 1.7% were positive for either the anti-GAD65 or the anti-ICA autoantibodies. There were 3.5% out of 12.1% that were specific for the anti-WRS autoantibodies (Figure 4E).

Taken together, among the anti-aaRS autoantibody-positive population, 10.3% were double positive for the anti-GAD65 and the anti-ICA autoantibodies, and 13.8% and 3.5% showed a single positive result for either the anti-GAD65 or anti-ICA antibodies, respectively. There were 10.3% out of 37.9% of the anti-aaRS autoantibody-positive population who showed specificity for anti-aaRS autoantibodies (Figure 4F). The anti-aaRS autoantibodies were found in 30% of subjects with type 1 DM (6 out of 20) who did not have the common classical type 1 DM autoantibodies, such as GAD65, IA-2 and ICA.

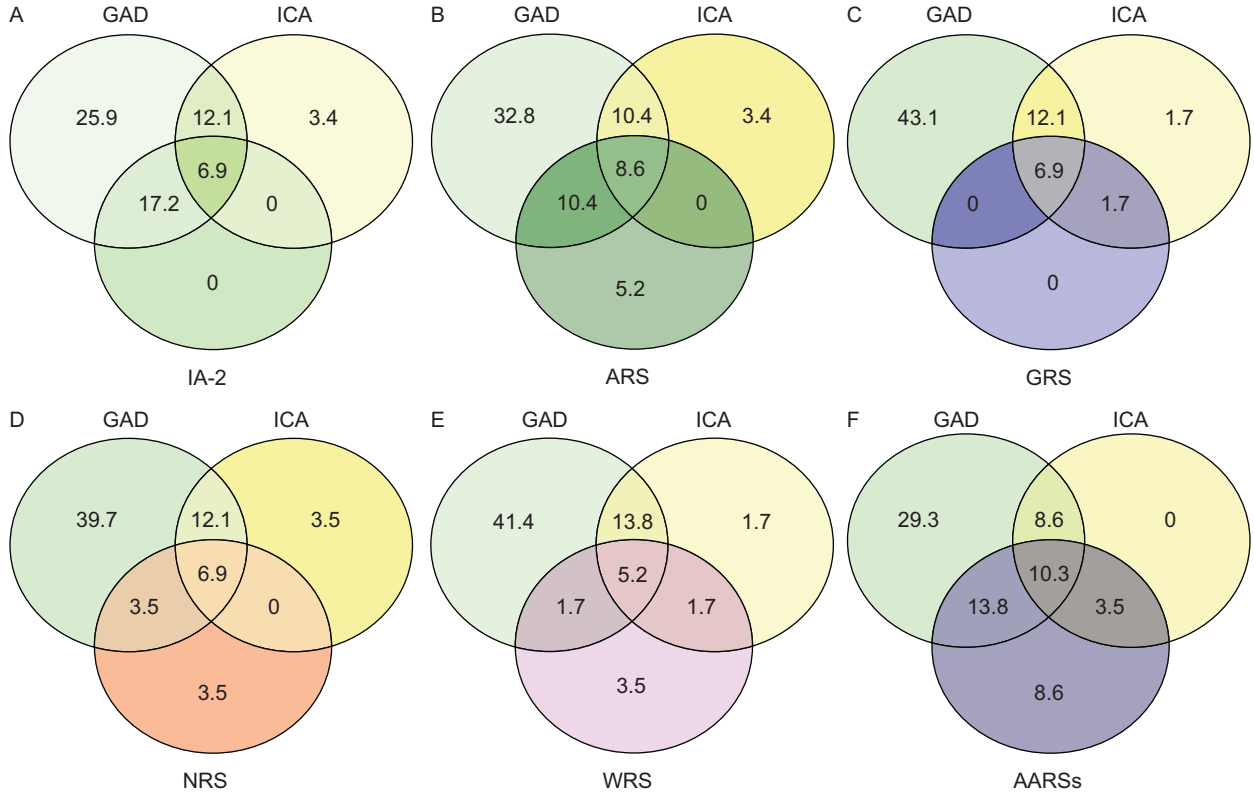

Figure 4. Differential analyses of anti-aminoacyl-tRNA synthetase (aaRS) autoantibodies in patients with type 1 diabetes mellitus (DM). (A) Autoantibodies against glutamic acid decarboxylase (GAD), islet cell antigen (ICA) and islet antigen 2 (IA-2) were detected with an enzyme-linked immunosorbent (ELISA) kit used according to the manufacturer's instructions. (B–F) Differential analyses of alanyl-tRNA synthetase (ARS), glycyl-tRNA synthetase (GRS), asparaginyl-tRNA synthetase (NRS) and tryptopohanyl-tRNA synthetase (WRS) compared with GAD, ICA and IA-2. Each specific position of the autoantibodies is described in the diagram.

Discussion

Many studies have recently reported that aaRSs are directly or indirectly involved in the development of a variety of diseases including the antisynthetase syndrome. In this study, the presence of autoantibodies against aaRSs in the plasma of diabetic patients was studied to determine if they could be used as a diagnostic marker for type 1 DM. Type 1 DM is caused by destruction of beta cells mediated by autoantigen reactive CD8+ T cells. WRS, GRS and NRS are known to be enriched in the pancreas and function as cytokine-recruiting immune cells (Boasso et al. 2005, Howard et al. 2002). The release of these molecules results in the apoptosis of pancreatic cells by attraction of autoreactive T cells, which leads to the development of type I DM. In addition, autoantibodies are thought to be produced for the protection of beta cells; this is achieved by the clearing of autoantigens. In order to confirm the production of anti-aaRS autoantibodies, the pancreatic localization of WRS, GRS, NRS and ARS was determined. Some aaRSs (ARS and GRS) were specifically enriched in the islets of Langerhans and others (NRS and WRS) are more evenly distributed throughout the pancreas. Although the major expression of aaRSs is not specific to beta cells, the generation of autoantibodies against aaRSs might be due to the fact that aaRSs are ubiquitously expressed in all tissue, even if in only small amounts, and they recruit autoreactive T cells. The analysis of human plasma revealed that anti-aaRS autoantibodies were significantly increased in patients with type 1 DM. However, the level of autoantibodies of many patients decreased after the onset of diabetes (Bilbao et al. 2000). Anti-aaRS autoantibodies were found in 30% of the subjects with type 1 DM without the common classical type 1 DM autoantibodies such as GAD65, IA-2 and ICA. These findings suggest that anti-aaRS autoantibodies might be useful as a diagnostic marker for type 1 DM.

The positive anti-aaRS autoantibody rate was 5.2% in patients with type 2 DM. About 5% of patients with type 2 DM who are positive for anti-GAD antibodies later develop type 1 diabetes (Kawasaki et al. 2006, Park et al. 1996). Therefore, patients with type 2 DM should be monitored for anti-aaRS antibodies because of the risk of developing autoimmune-related diseases such as type I DM.

Autoantibodies against aaRSs have been found in patients with inflammatory myopathy. In this study, however, patients with antisynthetase syndrome were not identified by the plasma analysis reactivity to aaRS antigens. The results of this study showed that some patients with type 1 DM who were positive for aaRS autoantibodies had other autoimmune diseases (myasthenia gravis, Graves disease, autoimmune haemolytic anaemia, hypothyroidism), while other patients had allergic rhinitis, diabetic Charcot joints or hearing loss. It is not surprising that type 1 DM is associated with other endocrine disorders (Riley 1992). The analysis of the human aaRS expression profile (http://symatlas.gnf.org/SymAtlas/) has shown that expression of ARS, GRS, NRS and WRS is enriched in the thyroid, brain and blood cells in addition to muscles, lungs and pancreas. Therefore, anti-aaRS-autoantibody generation appears to be associated with a variety of autoimmune diseases as well as the classical antisynthetase syndrome, including inflammatory myopathy, interstitial lung disease and arthritis.

Immunosuppressive drugs such as cyclosporin are used to suppress beta cell autoimmunity and rescue beta cell function (Bougneres et al. 1990). However, the therapeutic effects of these drugs are sustained only with continuous administration. Therefore, the possibility of immunotherapy using autoantigens has recently been studied as a method for the prevention of the development of type 1 DM (Alleva et al. 2002). Antigen-based immunotherapy has been used to establish beta cell-specific tolerance in NOD mice and in various mouse models of type 1 DM (Tisch et al. 1998, Casares et al. 2002). Therefore, further study of aaRSs, as a novel autoantigen, is required, to determine its role in the development of type 1 DM. Prevention of the development of type 1 DM by the induction of T-cell tolerance is one possible goal of immunotherapy; in addition, aaRSs might be used as a diagnostic marker of type 1 DM.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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