VOLUME 19, NUMBER 4

AUIMEI 19(4) 221–286 (1994)
ISSN 0891–6934

AUTOIMMUNITY

Contents

AUTOIMMUNITY is abstracted in: ISI (Current Contents®); Index Medicus; Excerpta Medica; Current Awareness in Biological Sciences (CABS); Current Science Ltd; Reference Update; Cambridge Science Abstracts and PASCAL, France.

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Autoimmunity, 1994, Vol. 19, pp. 231–238
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ASSOCIATION BETWEEN ANTIBODIES TO THE MR 67,000 ISOFORM OF GLUTAMATE DECARBOXYLASE (GAD) AND TYPE 1 (INSULIN-DEPENDENT) DIABETES MELLITUS WITH COEXISTING AUTOIMMUNE POLYENDOCRINE SYNDROME TYPE II

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(Received 1 September 1994; in final form 29 November 1994)

By using an immunoprecipitation assay, we analysed reactivity of autoantibodies to human recombinant GAD_{65} and GAD_{67} in sera from patients with autoimmune polyendocrine syndrome Type II (APS II) with and without Type 1 (insulin-dependent) diabetes mellitus (IDDM) compared to patients with organ-specific autoimmunity. Overall antibodies to GAD_{65} were correlated with IDDM in all study groups, whereas GAD_{67} antibodies were associated with IDDM when APS II coexists. Antibodies to GAD_{65} and GAD_{67} were detected in 13 (44.8%) and 7 (24.1%) out of 29 APS II patients with IDDM, but in only 4 (13.8%) and 2 (6.9%) out of 29 APS II patients without IDDM, respectively ($p < 0.05$). In short-standing IDDM (< 1 year), antibodies to GAD_{67} were significantly more frequent in patients with APS II (5 of 9 [55.6%] subjects) compared to matched diabetic patients without coexisting polyendocrinopathy (1 of 18 [5.6%] subjects) ($p < 0.02$). The levels of GAD_{65} (142 ± 90 AU) and GAD_{67} antibodies (178 ± 95 AU) were significantly higher in patients with polyglandular disease than in patients with isolated IDDM (91 ± 85 AU and 93 ± 57 AU) ($p < 0.02$). Interestingly, all 11 GAD_{67} antibody positive subjects also had GAD_{65} antibodies ($p < 0.0001$), and in 10 of 11 anti-GAD_{67} positive sera the GAD_{67} antibodies could be blocked by either GAD_{67} or GAD_{65}, suggesting the presence of cross-reactive autoantibodies. No correlation was observed between GAD antibodies and age, sex or any particular associated autoimmune disease, besides IDDM. GAD antibodies were present in only 1of 6 (16.7%) patients with APS Type I, in 1 of 26 (3.9%) patients with autoimmune thyroid disease but in none of the patients with Addison's disease (n = 16), pernicious anaemia (n = 7) or normal controls (n = 50). Our data suggest distinct antibody specificities reactive to GAD isoforms in APS II and IDDM, which might reflect different mechanisms of autoimmune response in IDDM with coexisting autoimmune polyendocrine autoimmunity.

KEY WORDS: Insulin-dependent diabetes mellitus, autoimmune polyendocrine syndrome, glutamic acid decarboxylase, autoimmunity.

INTRODUCTION

Autoimmune endocrinopathies are associated with lymphocytic infiltration and chronic destruction of the affected endocrine glands. The simultaneous involvement of the adrenal cortex, the thyroid, the pancreatic islets or the gastric parietal cells in one patient defines the autoimmune polyglandular endocrine syndrome Type II (APS II)[1–3]. In patients with APS II, a high prevalence of organ specific autoantibodies has been observed, including autoantibodies reactive to thyroperoxidase and thyroglobulin, adrenal 17-alpha hydroxylase or 21-hydroxylase and autoantibodies to the gastric proton pump H^+/K^+ ATPase, respectively[3,4].

In Type 1 (insulin-dependent) diabetes mellitus (IDDM) with and without coexisting autoimmunity, cytoplasmic islet cell antibodies (ICA) have been described as valuable serological marker[5,6]. Recently it has been demonstrated that ICA react with heterogenous islet antigens[7–9] including the enzyme glutamate decarboxylase (GAD), which has been previously identified as one target antigen of autoantibodies directed to the 64 KD islet cell protein[10]. Human GAD exists in two isoforms of Mr 65,000 (GAD_{65}) and Mr 67,000 (GAD_{67}) that are encoded by two distinct

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genes[11]. The detection of autoantibodies to GAD_{65} in 70–80% of patients with newly-diagnosed IDDM and prediabetic individuals has clearly demonstrated that GAD_{65} represents a major B-cell autoantigen in IDDM[12,13]. Apart from IDDM, high level GAD_{65} and GAD_{67} antibodies have been detected in Stiff-Man-Syndrome (SMS), a rare neurological disorder, where about 60% of the non-tumor associated form are correlated with organ-specific autoimmunity[14]. In SMS the humoral immunoreactivity to GAD is completely different from IDDM. The majority of GAD antibodies from SMS patients react with both isoforms including linear epitopes of GAD_{65}, whereas in IDDM the antibodies are predominantly directed to conformation epitopes of GAD_{65}[9,10,14]. Considering these data on the heterogeneity of the immune response to GAD in different disorders it is of interest to establish the possible significance of antibodies to both GAD isoforms in APS II, which is frequently associated with IDDM.

In this study, we examined the prevalence of antibodies to human GAD_{65} and GAD_{67} among patients with isolated organ-specific autoimmunity and subjects with APS. By means of an immunoprecipitation assay using human recombinant GAD proteins, we were able to demonstrate distinct patterns of autoantibody specificities in APS II and IDDM. Autoantibodies to GAD_{67} were mainly found in diabetic patients with APS II, which suggests differences in the autoimmune pathogenesis of IDDM with and without polyendocrine autoimmunity.

MATERIAL AND METHODS

Patients

Sera were studied from 58 patients with autoimmune polyendocrine syndrome Type II. APS II was defined by the presence of at least two of the following diseases: autoimmune Addison's disease, autoimmune thyroid disease, IDDM and pernicious anaemia, respectively. 29 of these patients had overt IDDM with a duration of the disease from 0 to 26 years (mean duration of IDDM 9.1 years). Furthermore, sera were obtained from 6 non-diabetic patients with autoimmune polyendocrine syndrome Type I (APS I). All patients with APS I had mucocutaneous candidiasis, adrenal insufficiency and hypoparathyroidism. Patients with clinical overt autoimmune disease affecting one endocrine tissue who were only positive for organ-specific autoantibodies to the respective endocrine gland served as controls. We tested 26 patients with autoimmune thyroid disease, including 14 patients with Hashimoto's thyroiditis and 12 patients with Graves' disease, 16 patients with autoimmune Addison's disease and 7 patients with pernicious anaemia. Sera from 18 patients with recent-onset IDDM (0–1 year) and 20 patients with long-standing IDDM (2–26 years) matched for age, sex and duration of disease were used as controls. Part of these sera had been previously characterized for ICA and GAD antibodies[12]. 50 healthy individuals without a family history of endocrine diseases served as controls. Sera were coded, stored at $-20°C$ and tested in a blinded way in all assays. Informed consent was obtained from all patients and control subjects.

Detection of autoantibodies to human GAD_{65} and GAD_{67}

Antigen expression in a baculovirus system and the preparation of ^{35}S-methionine labelled human recombinant GAD_{65} and GAD_{67} was performed as described recently[12,15]. Membrane fractions of the recombinant proteins containing equal amounts of radiolabelled proteins (1×10^6 cpm) were precipitated with normal human serum. Then the precleared extracts were incubated with 25 µl of test serum for 8 h and immunoprecipitated with Protein A Sepharose (Pharmacia, Freiburg). Samples were washed, eluted and fluorographed as described[12]. Gels were scanned by laser densitometry, the antibody levels were expressed in arbitrary units (AU) and calculated as follows: (unknown minus normal control)/(positive reference serum minus normal control) $\times$ 100. Sera were regarded as positive if GAD_{65} or GAD_{67} antibody levels were greater than 3 SD above the mean of 50 normal sera (9.3 AU and 11.8 AU, respectively). In the First IDW Proficiency Program for the standardisation of the GAD antibody assays, we achieved values of 100% for specificity, 83.3% sensitivity and 87,5% validity[16].

Blocking of GAD_{67} antibodies

To analyse cross-reactivity of GAD_{67} antibodies with GAD_{65} blocking experiment are performed in GAD_{67} antibody positive sera. Sera were incubated with membrane fractions of either unlabelled human recombinant GAD_{67} or GAD_{65} (50 µg/serum) for 4 h at room temperature. Noninfected Sf9 cells were used as control. After preincubation sera were subjected to immunoprecipitation as described above.

Immunofluorescence studies

ICA were analysed by indirect immunofluorescence testing on cryostat sections of the pancreas from a organ donor with blood group 0[17]. Results were expressed in JDF-Units (IDW Proficieny Program Lab ID no 116). The detection limit in our laboratory was 5 JDF-Units. Antibodies to parietal cells and to

adrenal cortex were detected by the standard indirect immunofluorescence test using cryostat sections of human stomach and human adrenal gland, respectively[17]. Thyroperoxidase and thyroglobin antibodies were determined using a commercial ELISA kit (ELIAS, Freiburg).

Statistic analyses

Statistical significance was determined by Chi-square test with Yates' correction or Fischer's exact test, where appropriate. Statistical differences among antibody levels were tested by Student's t-test. Antibody levels were expressed as mean ± SD.

RESULTS

The clinical and immunological characteristics of the study groups are summerized in Table 1. Overall antibodies to GAD_{65} and GAD_{67} were detected in 17 (29.3%) and 9 (15.5%) out of 58 patients with autoimmune polyendocrinopathy Type II (Figure 1). Our study revealed a significant association between antibodies to GAD and APS II when IDDM coexists. Antibodies to GAD_{65} and GAD_{67} were found in 13 (44.8%) and 7 (24.1%) out of 29 diabetic patients with APS II, but in only 4 (13.8%) and 2 (6.9%) out of 29 APS II patients without IDDM, respectively ($p < 0.05$) (Table 2). There was no correlation of GAD antibodies with age, sex and any particular associated organ-specific autoimmunity, besides IDDM (Table 3). Most strikingly, the presence of antibodies to GAD_{67} was related with IDDM as part of APS II. 5 of 9 (55.6%) patients with APS II and short-term IDDM (duration 0–1 year) had antibodies to GAD_{67} compared to 1 of 18 (5.6%) matched diabetic patients without coexisting endocrine autoimmune affection ($p < 0.01$) (Table 2). Within the study groups, the detection of antibodies to GAD_{67} was strongly correlated to GAD_{65} antibodies. All sera containing GAD_{67} antibodies were also positive for antibodies to GAD_{65} ($p < 0.0001$) (Table 3). By contrast, 8 of 58 (13.8%) patients with APS II and 12 of 38 (31.6%) patients with isolated IDDM had GAD antibodies which exclusively react with the GAD_{65} isoform. In blocking experiments GAD_{67} antibodies could be completely preabsorbed with equal amounts of either GAD_{67} or GAD_{65} in 8 of the 9 (88.9%) GAD_{67} antibody positive patients with polyendocrine autoimmunity and in two patients with monoglandular IDDM. Preincubation with GAD_{65} resulted in a significant reduction but not a complete blocking of GAD_{67} reactivity in one anti-GAD_{67} positive serum (serum No. 13) (Figure 2). As shown for GAD_{67} antibodies, antibodies to GAD_{65} were increased in patients with APS II and a short duration of IDDM (7 of 9 [77.8%] versus 10 of 18 [55.6%]) (p = n.s.). In addition, GAD_{65} antibodies were associated with the presence of ICA (ICA in 9 of 13 [69.2%] patients with APS II and IDDM, $p < 0.02$ and in 4 of 4 [100%] patients with APS II without IDDM, $p < 0.0001$). The levels of antibodies to GAD_{65} (142 ± 90 AU) and GAD_{67} (178 ± 95 AU) were significantly higher in polyendocrine patients with IDDM compared to patients with isolated IDDM (91 ± 85 AU and 93 ± 52 AU) ($p < 0.02$). Interestingly, we also found high level GAD_{65} antibodies in 4 sera from non-diabetic patients with APS II (183 ± 77 AU) (Table 3).

Among the controls only 1 out of 6 (16.7%) patients with APS I and 1 of 26 (3.9%) subjects with autoimmune thyroid disease, a 50 year old male with Graves' disease, were found GAD_{65} antibody positive (Fig. 1A lane 7) ($p < 0.005$). None of the patients with autoimmune Addison's disease ($p < 0.01$), pernicious anaemia ($p < 0.05$) or of normal subjects ($p < 0.0001$) had antibodies to GAD_{65} or GAD_{67} (Table 2).

Table 1 Characterisation of study groups

Study groups	Number of sera	Sex (F/M)	Age		Autoantibodies			
			Mean	Range	TGA	TMA	ACA	PCA
APS II								
without IDDM	29	20/9	41.0	18–68	21	22	18	16
with IDDM	29	19/10	28.4	13–62	21	20	10	10
APS I	6	4/2	17.5	12–22	1	1	3	2
IDDM	38	26/12	24.3	13–62	0	0	0	0
Autoimmune thyroid	26	16/10	35.3	13–58	23	26	0	0
Autoimmune Addison's	16	7/9	34.2	9–56	0	0	16	0
Pernicious anaemia	7	2/5	38.6	20–50	0	0	0	7
Controls	50	28/22	25.6	8–38	0	0	0	0

IDDM, insulin-dependent diabetes mellitus; APS II, autoimmune polyendocrine syndrome Type II; APS I, autoimmune polyendocrine syndrome Type I. TGA, thyroglobulin antibodies; TMA, thyroid microsomal antibodies; ACA, adrenocortical antibodies; PCA, parietal cell antibodies.

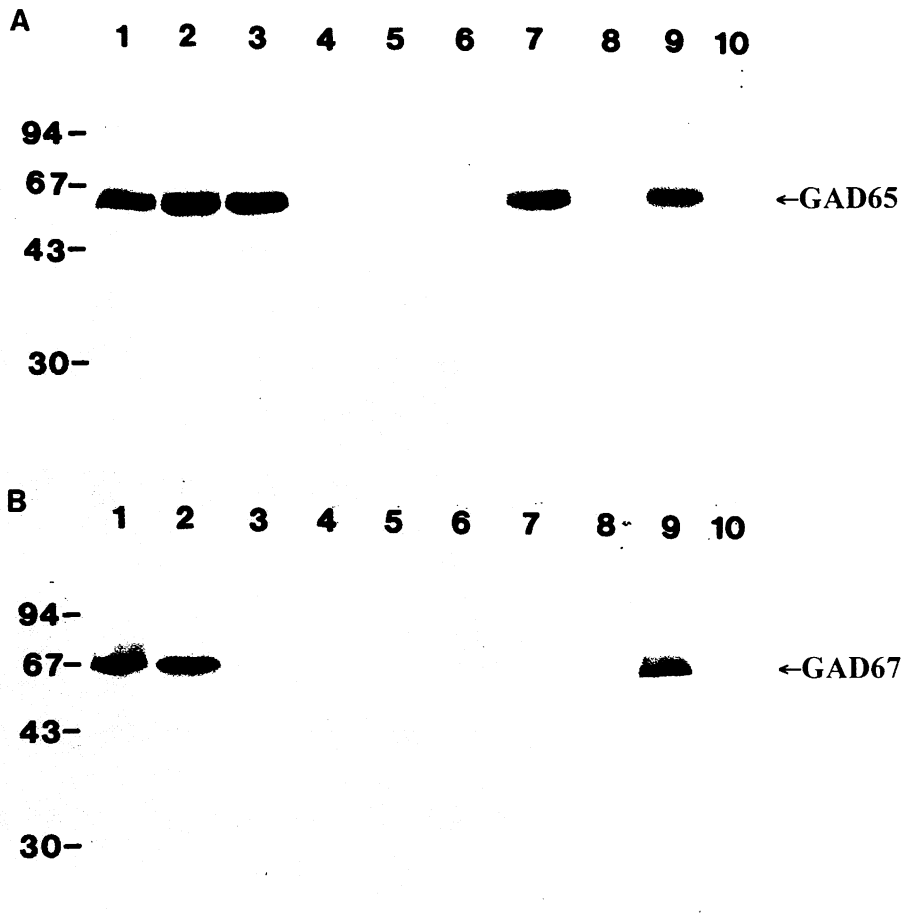

Figure 1 Immunoprecipitation of human recombinant GAD_{65} and GAD_{67}. [^{35}S]-methionine labelled GAD_{65} (A) and GAD_{67} (B) were immunoprecipitated with sera from APS II patients with IDDM (lanes 1 and 3), APS II without IDDM (lanes 2 and 4), Addison's disease (lane 5), pernicious anaemia (lane 6), autoimmune thyroid disease (lanes 7 and 8), and a normal control subject (lane 10). As positive controls the immunoprecipitation of mouse monoclonal antibody GAD 1 (A lane 9) and the rabbit antiserum K-2 (B lane 9) is demonstrated. Molecular weight markers ($Mr \times 10^{-3}$) are indicated on the left margin.

DISCUSSION

The association of IDDM with Graves' disease, Hashimoto's thyroiditis and Addison's disease has been extensively documented[1,2,18]. In patients with APS II a multitude of organ-specific autoantibodies has been described indicating latent or overt autoimmune diseases. Studies in IDDM without coexisting organ-specific autoimmunity have shown that only the small isoform of GAD, GAD_{65}, represents a major target antigen, whereas the detection of antibodies to GAD_{67} apparently does not provide additional information[12,13].

In this study, we demonstrate for the first time significant differences in autoantibody reactivity against human GAD_{65} and GAD_{67} in patients with immunological and clinical features of APS II compared to diabetic patients without polyendocrinopathy. 56% of patients with autoimmune polyendocrine autoimmunity had antibodies to GAD_{67} at the onset of IDDM, whereas these antibodies were rare in isolated IDDM. In new onset IDDM GAD_{67} antibodies have been reported in 9–18% of the cases[12,13], but in those studies a discrimination between mono- and polyglandular failure was not performed. Since polyendocrine autoimmunity in IDDM is not uncommon we have carefully reexamined nine GAD_{67} antibody positive patients with IDDM from a previous study[12] for the simultaneous presence of diabetes unrelated organ-specific autoantibodies. Autoantibodies to thyroid, adrenal cortex or gastric parietal cells were found in six of these nine subjects (data not shown). Thus, only a few cases have GAD_{67} antibodies without detectable associated organ-specific antibodies. It is

Table 2 Prevalence of antibodies to GAD_{65} and GAD_{67} in patients with APS II and organ-specific autoimmune diseases

Autoimmune manifestation	Number of sera	Autoantibodies		
		GAD_{65}-Ab	GAD_{67}-Ab	ICA
APS II				
without IDDM	29	4 (13.8%)	2 (6.9%)	4 (13.8%)
with IDDM	29	13 (44.8%)*	7 (24.1%)	12 (41.4%)
duration 0–1 year	9	7 (77.8%)**	5 (55.6%)#	7 (77.8%)
duration > 1 year (mean 12.5 years)	20	6 (30.0%)	2 (10.0%)	5 (25.0%)
APS I	6	1 (16.7%)	0	1 (16.7%)
IDDM[a]				
duration 0–1 year	18	10 (55.5%)	1 (5.5%)	11 (61.1%)
duration > 1 year	20	4 (20.0%)	1 (5.0%)	1 (5.0%)
Thyroid disease	26	1 (3.9%)	0	0
Addison's disease	16	0	0	0
Pernicious anaemia	7	0	0	0
Controls	50	0	0	0

ICA, islet cell antibodies; GAD_{65}-Ab, antibodies to GAD_{65}; GAD_{67}-Ab, antibodies to GAD_{67}; IDDM, insulin-dependent diabetes mellitus; APS II, autoimmune polyendocrine syndrome Type II; APS I, autoimmune polyendocrine syndrome Type I; [a]patients with isolated IDDM were matched for age, sex and duration of with respect to APS II patients with IDDM; *$p < 0.05$, ** $p < 0.002$ vs APS II without IDDM; #$p < 0.01$ vs isolated IDDM.

Table 3 Clinical and immunological characteristics of GAD antibody positive patients with autoimmune polyendocrine syndrome Type II. GAD antibody levels are expressed in arbitrary units (AU)

Patients No.	Age Sex	Autoimmune disease	Duration of IDDM (years)	Autoantibodies						
				GAD_{65}-Ab (AU)	GAD_{67}-Ab (AU)	ICA (JDF-U)	TGA (IU/ml)	TMA (IU/ml)	ACA	PCA
1	62F	DM, AD	2	16	–	–	438	1031	+ +	–
2	42F	DM, AD, GD	1	236	17	80	1111	1368	+	–
3	25F	DM, HT	0	44	–	20	507	3000	–	–
4	33M	DM, GD	0	260	178	160	–	1299	+	–
5	20M	DM, AD	4	28	–	–	–	–	+	+
6	57M	DM, PD	25	290	268	20	3000	532	–	+
7	34F	DM, HT	2	66	–	–	3381	3000	+	–
8	28F	DM, GD	4	90	–	–	622	2640	–	–
9	16F	DM, HT, PD	1	138	278	10	3779	957	–	+ +
10	17F	DM, AD	0	211	247	80	–	–	+	+
11	22F	DM, AD	0	177	–	80	–	–	+	–
12	20F	DM, AD, HT	10	132	112	320	3559	3000	+	+
13	18F	DM, AD, HT	0	158	149	160	2869	2735	+	–
14	65F	GD, PD	–	150	–	80	587	1832	–	+ +
15	35F	AD, HT	–	100	–	20	3217	3000	+	–
16	60F	AD, GD	–	280	70	40	670	1878	+	–
17	53F	AD, HT, PD	–	200	113	160	731	2847	+	+

DM, insulin-dependent diabetes mellitus; AD, Addison's disease; HT, Hasimoto's thyroiditis; GD, Graves' disease; PD, pernicious disease; GAD_{65}-Ab, antibodies to GAD_{65}; GAD_{67}-Ab, antibodies to GAD_{67}; ICA, islet cell antibodies; TGA, thyroglobulin antibodies; TMA, thyroid microsomal antibodies; ACA, adrenocortical antibodies; PCA, parietal cell antibodies; JDF-U, Juvenile Diabetes Foundation Units; +, positive; + +, strongly positive; –, negative.

worth noting that in this study we examined only autoantibodies to the most frequently involved endocrine tissues, suggesting that in some of these anti-GAD_{67} positive sera other autoantibodies may coexist. In striking contrast to a recent study, which reported on antibodies to GAD_{65} in 5 of 5 and antibodies to GAD_{67} in 2 of 5 non-diabetic patients with APS I, we here found no association between GAD antibodies and this condition[19,20]. The clinical features of the subjects (age, sex and symtoms of APS I: mucocutaneous candidiasis, hypoparathyroidism and Addison's disease) seem to be similar to our patients

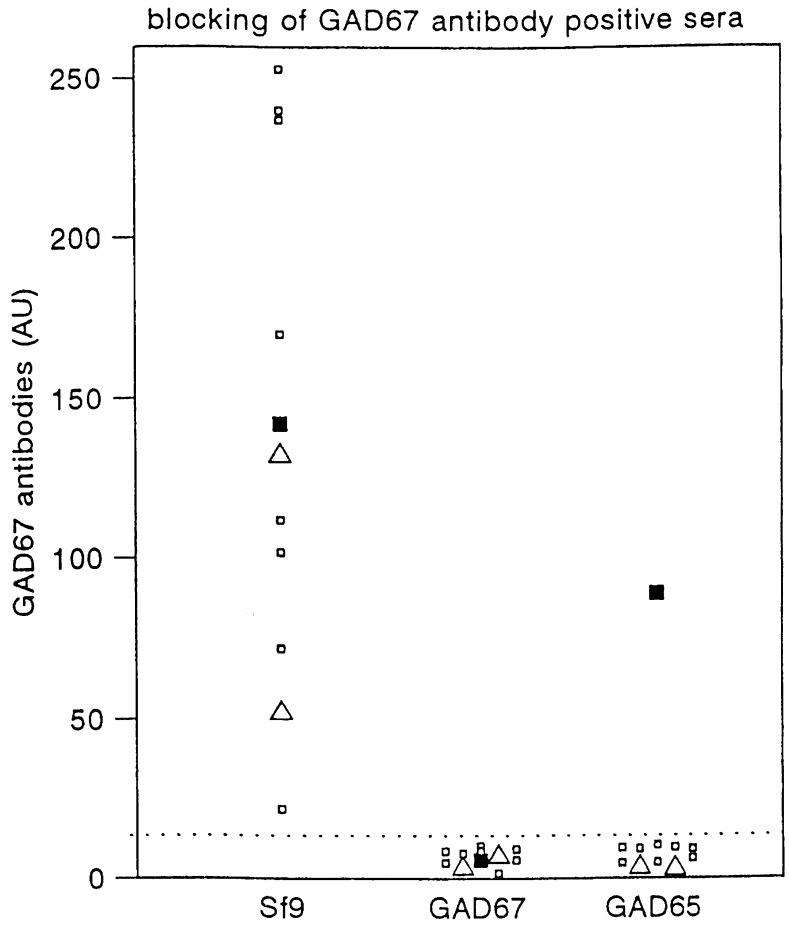

Figure 2 Blocking of antibodies to GAD_{67} in patients with autoimmune polyendocrine syndrome Type II (□) and patients with isolated IDDM (△). GAD_{67} antibody positive sera were preincubated with membrane fractions of unlabelled Sf9 cells, GAD_{67}, or GAD_{65}, and then analysed by immunoprecipitation test. ■, serum which was only partly blocked by preincubation with GAD65. The antibody levels were expressed in arbitrary units (AU). The dashed line represents the cut-off (11.8 AU) for the GAD_{67} antibodies (mean + 3SD of 50 normal controls).

but the immunological characteristics differ significantly. In the previous study all patients were detected as ICA positive[20], whereas we here observed ICA only in one patient with APS I, which is consistent with previous studies reported on ICA in only a minority of APS I patients[1,2]. Thus, we suggest that the high frequency of GAD antibodies is explained by a selection of ICA positive patients. Since GAD antibodies contribute to ICA reactivity[7–9] this would result in an overestimation of the prevalence of GAD antibodies. However, due to the small number of patients with APS I in both studies, which do not allow appropriate statistical analysis, it is premature to draw final conclusions as to the role of GAD antibodies in this syndrome.

The increased prevalence of GAD_{67} antibodies in APS II, observed in this study, could be explained by a primary autoimmune response directed to the GAD_{67} isoform. Immuno-precipitation, Western blot and Northern blot analysis have clearly shown that human islets contain only GAD_{65}[11,21]. In contrast, GAD_{67} is widely expressed in the human central nervous system (CNS) and in some peripherial organs, e.g. adrenal and testis[22]. Thus, the antibody response to GAD_{67} could be initiated by the autoimmune affection of the above mentioned tissues. The adrenal glands, gonads, the pituitary and the hypothalamus can all represent targets in polyendocrine autoimmunity[2,3]. Although it cannot be excluded that GAD_{67} is directly involved in polyendocrine autoimmunity, our data suggest that the immune recognition of GAD_{67} may be secondary to humoral GAD_{65} autoimmunity. We found higher prevalances of antibodies to GAD_{65} than antibodies to GAD_{67} and a restriction of GAD_{67} antibodies to GAD_{65} antibody positive individuals. Most importantly, we were able to demonstrate that in all but one sera GAD_{67} antibodies could be completely blocked by preincubation with either GAD_{67} or GAD_{65} sug-

gesting that in the majority of polyendocrine patients GAD_{67} antibodies are directed to common epitopes of both isoforms. These data agree with those obtained in isolated IDDM[12,13,23] and, thus strongly suggest that GAD_{65} also represents a major target antigen in cases where IDDM is part of APS II. Consistent with our data a high prevalence of GAD antibodies has been reported in patients with IDDM and autoimmune thyroid disease, but in that study the isoform specificities were not assessed[24].

We here confirmed an association of GAD antibodies with IDDM, however, we also detected four non-diabetic patients with polyglandular syndrome and high level GAD antibodies. This findings could be explained by a preclinical state of IDDM, but it is more likely that in polyendocrine patients GAD antibodies can occur without autoimmune affection of the islets of Langerhans. Indeed, a longitudinal study has recently shown that some GAD antibody positive non-diabetic subjects with polyendocrine autoimmunity do not develop Type 1 diabetes[25]. Furthermore, immunohistochemical studies failed to detect signs of insulitis in three GAD antibody positive patients with APS II[26]. These data support the hypothesis that in APS II the autoimmune response can be directed to GAD expressed in non-islet cell endocrine or neuronal tissues.

In this study we were able to demonstrate a heterogeneous humoral autoimmune response directed to human GAD isoforms in diabetic patients with monoglandular disease or coexistent autoimmune polyendocrinopathy. Our data suggest that the discrimination of antibody reactivities to GAD_{65} and GAD_{67} may provide information as to the potential coexistence of or progression towards polyglandular failure in patients with IDDM. Obviously, a larger number of patients with APS II and longitudinal studies are required to clarify common and diverse mechanisms of autoimmunity to GAD_{65} and GAD_{67} in different conditions. These studies may be important for a better understanding of the role of GAD autoimmunity in IDDM.

Acknowledgments

The study was supported by grants of the Deutsche Forschungsgemeinschaft Se 725/1-1 (J.S.), Sche 225/6-3 (W.A.S) and BO 829/6-1 (B.O.B.), the Deutsche Diabetesstiftung (J.S.), and the Juvenile Diabetes Foundation International (W.A.S.).

References

1. Bottazzo G.F. and Doniach D. Polyendocrine autoimmunity: an extended concept In: *Autoimmunity and endocrine disease*, ed. Volpe, R., Marcel/Dekker Inc. New York, 1985 pp. 375–403.
2. Bottazzo G.F., Todd I., Mirakian R., Belfiore A. and Pujol-Borrell R. Organ-specific autoimmunity. A 1986 overview. *Immunol. Rev.* 1986: **94**: 137–169.
3. Muir A. and MacLaren N.K. Autoimmune diseases of the adrenal glands, parathyroid glands, gonads, and hypothalamic-pituitary axis. *Endorin. Metab. Clin.* 1993; **20**: 619–643.
4. Banga J.P., Barnett P.S., Mahadevan D. and McGregor A.M. Immune recognition of antigen and its relevance to autoimmune disease: recent advances on the molecular level. *Europ. J. Clin. Invest.* 1989; **19**: 107–116.
5. Tarn A.C., Thomas J.M., Dean B.M., *et al.* Predicting insulin-dependent diabetes. *Lancet* 1988; 845–850.
6. Riley W.J., MacLaren N.K., Kirscher J., Spillar R.P., Silverstein J.H., Schatz D.A., *et al.* A prospective study of the development of diabetes in relatives of patients with insulin-dependent diabetes. *N. Engl. J. Med.* 1990; **323**: 1167–1172.
7. Genovese S., Bonifacio E., McNally J.M., Dean B.M., Wagner R., Bosi E., *et al.* Distinct cytoplasmic islet cell antibodies with different risks for type 1 (insulin-dependent) diabetes mellitus. *Diabetologia.* 1992; **35**: 385–388.
8. Scherbaum W.A. Cytoplasmic islet cell antibodies (ICA): towards a molecular understanding of the autoantigens. *Clin. Endocrin.* 1994; **40**: 15–18.
9. Richter W., Seissler J., Northemann W., Wolfahrt S., Meinck H.M. and Scherbaum W.A. Cytoplasmic islet cell antibodies recognize distinct antigens in IDDM but not in Stiff Man Syndrome. *Diabetes* 1993; **42**: 1642–1648.
10. Baekkeskov S., Aanstoot A.J., Christgau S., Reetz A., Solimena M. and Cascalho M., *et al.* Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* 1990; **347**: 151–156.
11. Karlsen A.E., Hagopian W.A., Grubin C.E., Dube S., Distech C.M., Adler D.A., *et al.* Cloning and primary structure of a human islet isoform of glutamic acid decarboxylase from chromosome 10. *Proc. Natl Acad. Sci.* 1991; **88**: 8337–8341.
12. Seissler J., Amann J., Mauch L., Haubruck H., Wolfahrt S., Bieg S., *et al.* Prevalence of autoantibodies to the 65- and 67-kD isoforms of glutamate decarboxylase in insulin-dependent diabetes mellitus. *J. Clin. Invest.* 1993; **92**: 1394–1399.
13. Hagopan W.A., Michelsen B., Karlsen A.E., Larsen F., Moody A., Grubin C.E., *et al.* Autoantibodies in IDDM primarily recognize the 65,000-Mr rather than the 67,000-Mr isoform of glutamic acid decarboxylase. *Diabetes* 1993; **42**: 631–636.
14. Solimena M., Folli F., Aparisi R., Pozza G. and De Camilli P. Autoantibodies to GABA-eric neurons and pancreatic beta cells in Stiff-Man Syndrome. *N. Engl. J. Med.* 1990; **322**: 1555–1560.
15. Mauch L., Seissler J., Haubruck H., Cook N., Abney C.C., Berthold H., *et al.* Baculovirus mediated expression of human 65a and 67 KDa glutamic acid decarboxylase in SF9 insect cells and their relevance in diagnosis of insulin-dependent diabetes mellitus. *J. Biochem.* 1993; **113**: 699–704.
16. Schmidli RTS., Colman P.G., Bonifacio E., Bottazzo G.F. and Harrison L.C. High level of concordance between assays for glutamic acid decarboxylase antibodies. The first interbational glutamic acid decarboxylase antibody workshop. *Diabetes* 1994; **43**: 1005–1009.
17. Scherbaum W.A., Mirakian A.R., Pujol-Borrell R., Dean M. and Bottazzo G.F. Immunochemistry in the study and diagnosis of organ-specific autoimmune diseases. In: Polak J.M., Van Noorden S. (eds) Immunochemistry. Modern Methods and Applications. Wirght Bistrol 1986: pp 456–476.
18. Betterle C.F., Zanette F., Pedini B., Presotto F., Rapp L.B.,

Monciotto C.M., *et al.* Clinical and subclinical organ-specific autoimmune manifestations in Type 1 (insulin-dependent) diabetic patients and their first degree relatives. *Diabetologia.* 1984; **26**: 431–436.
19. Björk E., Velloso L.A., Kämpe O. and Karlsson F.A. GAD autoantibodies in IDDM. Stiff-Man Syndrome and autoimmune polyendocrine syndrome Type I recognize different epitopes. *Diabetes* 1993; **43**: 161–165.
20. Velloso L.A., Winqvist O., Gustafsson J., Kämpe O. and Karlsson F.A. Autoantibodies against a novel 51 KDa islet antigen and glutamate decarboxylase isoforms in autoimmune polyendocrine syndrome Type 1. *Diabetologia.* 1994; **37**: 61–69.
21. Kim J., Richter W., Aanstoot H.J. and Beakkeskov S. Differential expression of GAD_{65} and GAD_{67} in human, rat, and mouse pancreatic islets. *Diabetes* 1993; **42**: 1799–1808.
22. Christie M.R., Brown T.J. and Cassidy D. Binding of autoantibodies in sera from Type 1 (insulin-dependent) diabetic patients to glutamate decarboxylase from rat tissues. Evidence for antigenic and non-antigenic forms of the enzyme. *Diabetologia.* 1992; **35**: 380–384.
23. Ujihara N., Daw K., Gianini R., Boel E., Yu L. and Powers A.C. Identification of glutamic acid decarboxylase autoantibody heterogeneity and epitope regions in Type 1 diabetes. *Diabetes* 1994; **43**: 968–975.
24. Kawasaki E., Takino H., Yano M., Uotani S., Matsumoto K., Takao Y., *et al.* Autoantibodies to glutamic acid decarboxylase in patients with IDDM and autoimmune thyroid disease. *Diabetes* 1994; **43**: 80–86.S.
25. Wagner R., Genovese S., Bosi E., Becker F., Bingly P.J., Bonifacio E., *et al.* Slow metabolic deterioration towards diabetes in islet cell antibody positive patients with autoimmune polyendocrine disease. *Diabetologia.* 1994; **37**: 365–371.
26. Wagner R., McNally J.M., Bonifacio E., Genovese S., Foulis A., McGill M., *et al.* Lack of immunohistochemical changes in the islets of nondiabetic, autoimmune, polyendocrine patients with β-selective GAD-specific islet cell antibodies. *Diabetes* 1994; **43**: 851–856.