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GAD antibody-associated neurological illness and its relationship to gluten sensitivity

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Background – The high prevalence of gluten sensitivity in patients with stiff-person syndrome (SPS) lead us to investigate the relationship between gluten sensitivity and GAD-antibody-associated diseases. *Methods* – We used ELISA assays for anti-GAD and for serological markers of gluten sensitivity. Patients were recruited from clinics based at the Royal Hallamshire hospital, Sheffield, UK. Patients with gluten sensitivity were followed up after the introduction of a gluten-free diet and serological testing was repeated. *Results* – Six of seven (86%) patients with SPS were positive for anti-GAD, mean titre 109 U/ml; This compared with 9/90 (11%) patients with idiopathic sporadic ataxia, mean titre 32 U/ml, 16/40 (40%) patients with gluten ataxia, mean titre 25 U/ml, and 6/10 patients with type 1 diabetes only, mean titre 8 U/ml. None of 32 patients with celiac disease only, and of 40 patients with genetic ataxia were positive for anti-GAD. The titre of anti-GAD reduced following the introduction of a gluten-free diet in patients with SPS who had serological evidence of gluten sensitivity. The same was observed in patients with gluten ataxia and anti-GAD antibodies. This was also associated with clinical improvement. *Conclusion* – These findings suggest a link between gluten sensitivity and GAD antibody-associated diseases.

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Introduction

Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme in the synthesis of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). GAD is found in both the central and peripheral nervous systems (including the enteric nervous system) as well as in pancreatic beta cells (1). Antibodies against pancreatic islet cell proteins were first detected in children with insulin-dependent diabetes mellitus (IDDM) and were subsequently characterized as GAD antibodies (2–4). The first neurological disease to be associated with GAD antibodies was stiff-person syndrome (SPS) (5). GAD antibodies have been shown to be present in the sera of 60% of patients with SPS, 80% of patients with IDDM, in patients with polyendocrine autoimmune syndromes and in some cases of sporadic idiopathic ataxia (6–11). There is evidence to suggest that the pathogenesis of SPS may be a direct consequence of antibody-mediated neuronal dysfunction (12). However, it is not yet confirmed that GAD antibodies are directly responsible because, first GAD is an intracellular enzyme, and secondly up to 40% of patients with SPS do not have GAD antibodies (6).

Potential cell surface antigens recognized by pathogenic autoantibodies remain elusive but some candidates have recently been identified, including amphiphysin in paraneoplastic SPS (13), and $GABA_A$ receptor-associated protein in idiopathic SPS (14).

Gluten sensitivity is an autoimmune disease with diverse manifestations. Our personal experience of a high prevalence of antigliadin antibodies in patients with SPS and the overlap in the

neurological presentation between gluten sensitivity and GAD antibody-associated diseases, lead us to investigated further a possible relationship between the two.

Methods

Patients were recruited from the gluten sensitivity/neurology, coeliac disease ataxia and diabetes clinics, Royal Hallamshire Hospital, Sheffield, UK. The study was approved by the South Sheffield Research Ethics Committee.

Group 1 consisted of seven patients with clinical features and neurophysiological evidence of SPS (6). Sera were collected at diagnosis and for those patients with gluten sensitivity, repeated after starting a gluten-free diet (mean duration of the diet 3.3, range 1–7 years). Group 2 consisted of 90 patients with idiopathic sporadic ataxia. Group 3 consisted of 40 patients with gluten ataxia (idiopathic sporadic ataxia with positive antigliadin antibodies). The serological testing on these patients was performed at baseline and like in group 1 was repeated whilst on a gluten-free diet (mean duration of the diet 2.2 years, range 1–5 years). Group 4 consisted of 32 patients with gluten sensitive enteropathy (coeliac disease-CD). All of these 32 patients had the characteristic triad of villous atrophy, crypt hyperplasia and increase in the intraepithelial lymphocytes on duodenal biopsy. Samples from these patients were collected at baseline (prior to the introduction of a gluten-free diet). Group 5 consisted of 10 patients with type 1 diabetes. None of the patients in the last two groups (groups 4 and 5) had any evidence of neurological disease. Finally, group 6 consisted of 40 patients with genetically characterized ataxia.

GAD antibodies were estimated using an enzyme-linked immunosorbent assay kit (RSR, Cardiff, UK). According to the manufacturers instructions, a positive result is indicated by a value of >1 U/ml. This assay has been shown to have a sensitivity and specificity identical to radioimmunoassays (15, 16).

Antigliadin IgA and IgG antibodies were performed by the regional immunology laboratory using a commercially available ELISA kit. In addition to these antibodies, serum from patients with SPS was tested for transglutaminase (TG2 and TG6) antibodies as previously described (17) and for deamidated gliadin antibodies using Quanta Lite assays (INOVA Diagnostics Inc, San Diego, CA, USA) according to the manufacturer's instructions. Patients with positive serology also underwent duodenal biopsy to establish the presence of enteropathy.

Results

Prevalence of GAD antibodies in the selected patient groups

The prevalence of GAD antibodies for each group was as follows: group 1 (SPS) 6/7 (86%). The mean titre at diagnosis for those patients positive for GAD antibodies was 109 U/ml (range 4–190). Group 2 (idiopathic sporadic ataxia group) 9/90 (10%). The mean GAD antibody titre for the positive patients in this group was 32 U/ml (range 3–108). Group 3 (patients with gluten ataxia) 16/40 (40%). The mean GAD antibody titre for the positive patients in this group was 25 U/ml (range 2–130). Group 4 (patients with CD only), none of the 32 patients in this group had detectable GAD antibodies. Group 5 (patients with type 1 diabetes and no neurological illness) 6/10 (60%). The mean GAD antibody titre for the positive patients in this group was 8 U/ml (range 3–20). None of the 40 patients from group 6 (genetic ataxias) had detectable levels of GAD antibodies. These results are summarized in Table 1. The prevalence of anti-GAD antibodies in each of SPS, GA and IDDM groups was significantly higher than in the combined control groups ($P < 0.0001$, chi-squared test). The baseline anti-GAD antibody titre in the SPS group was significantly higher than in the gluten ataxia group ($P = 0.0042$, ANOVA).

Evidence of gluten sensitivity in SPS patients

Initial screening using antigliadin antibodies showed that five of the seven patients with SPS were positive for antigliadin antibodies. Further evidence of gluten sensitivity was therefore looked

Table 1 Prevalence and titres of GAD antibodies in the groups studied. The prevalence was significantly higher in each of groups 1, 3 and 5 when compared with the combined control groups ($P < 0.0001$, chi-squared test)

Patient group and type of illness/control (no. patients)	Group 1 Stiff person syndrome (7)	Group 2 Idiopathic sporadic ataxia (90)	Group 3 Gluten ataxia (40)	Group 4 Coeliac disease only (32)	Group 5 Type 1 diabetes only (10)	Group 6 Genetic ataxias (40)
Prevalence of GAD antibody	6/7 (86%)	9/90 (10%)	16/40 (40%)	0/32 (0%)	6/10 (60%)	0/40 (0%)
Anti-GAD titre Mean (range) U/ml	109 (4–190)	32 (3–108)	25 (2–130)	0	8 (3–20)	0

Table 2 Evidence of gluten sensitivity with or without enetropathy (CD) in seven patients with stiff-person syndrome (SPS). In total, six of seven patients had serological evidence of gluten sensitivity. All six had the HLA DQ2 found in >90% of patients with celiac disease (CD)

Patient with SPS no.	HLA type	Antigliadin antibodies		Anti-TG2 IgA >56 pos	Deamidated gliadin antibodies >20 units pos	AntiTG6		Gluten sensitivity	CD
		IgG	IgA			IgG >50 pos	IgA >56 pos		
1	DQ2	pos	neg	45	8	**72**	**104**	Yes	No
2	DQ2	pos	neg	33	19	42	**78**	Yes	No
3	DQ2	pos	pos	**129**	**128**	23	**87**	Yes	Yes
4	DQ2	pos	neg	2	8	**56**	33	Yes	No
5	DQ2	neg	neg	29	8	**59**	**76**	Yes	No
6	DQ1	neg	neg	20	7	40	26	No	No
7	DQ2	neg	neg	**60**	4	30	**78**	Yes	No

Bold are the values that are positive.

for in all seven patients. This included serological testing for transglutaminase 2 (IgA), 6 (IgG and IgA) and deamidated gliadin antibodies, HLA typing and duodenal biopsies to look for any evidence of enteropathy. A summary of the findings is shown in Table 2. Six of the seven patients had evidence of gluten sensitivity suggesting an overlap with SPS. All six patients had the HLA DQ2 which is seen in over 90% of patients with coeliac disease. Duodenal biopsy revealed enteropathy in just one of the six patients. Interestingly, all six patients had antibodies to TG6 while only two tested positive for TG2 IgA and one for deamidated gliadin, respectively. One of these two patients was the one with enteropathy and the other had evidence of malabsorption (folate, B12 and copper deficiency) but a normal duodenal biopsy. The high positivity of TG6 antibodies is in line with the recent finding of an immune response primarily targeting TG6 in patients with neurological manifestations of gluten sensitivity (18).

Clinical and serological effect of gluten-free diet on those patients with GAD antibodies and suspected gluten sensitivity

The effect of gluten-free diet on the GAD antibody titre in all patients with positive GAD antibodies and gluten sensitivity is shown on Table 3. The table also shows any additional autoimmune diseases in patients with positive GAD antibodies. The clinical characteristics of the seven patients with SPS are as follows: the group consisted of three male and four female patients, mean age of 51 (range 35–70). Of the six patients with SPS and gluten sensitivity, four went onto a strict gluten-free diet with evidence of elimination of the antigliadin antibodies, implying strict adherence to the diet. The diet was introduced without any alteration to their symptomatic anti-spasmotic medication. None of these patients were receiving any immunomodulatory medication (e.g. intravenous immunoglobulins). Two of the four patients that went on a gluten-free diet only managed to continue the diet for 1 year despite a degree of clinical stability. There was a reduction of the titre of GAD antibodies in all four patients with positive GAD antibodies and gluten sensitivity whilst on a gluten-free diet. In those two patients who stopped the diet at 1 year, the titre increased. One of these patients is much worse, currently requiring treatment with intravenous immunoglobulins. The other is stable just on antispasmodic medication. Clinical improvement that was sustained was seen in the two patients who persisted with the gluten free diet. These patients have not required any alteration of their pharmacological symptomatic treatment or immunosuppressive therapy. In the patient that has been on the diet the longest (7 years) with stability, sequential serological testing showed progressive reduction of the GAD antibodies as follows: 190 U/ml at baseline in 2001, 150 U/ml in 2005 to 115 U/ml in 2006, to 89 U/ml in 2008.

The ataxia in patients with gluten ataxia has already been shown to stabilize or improve following a strict gluten-free diet (18). Of the 16 patients with gluten ataxia and positive GAD antibodies, 11 patients went onto a strict gluten free diet whilst five patients refused the diet. In patients with gluten ataxia, the gluten-free diet resulted in complete elimination of the GAD antibodies in 10/11 patients and dramatic reduction in the GAD antibody titre in the remaining patient. There was clinical improvement of the ataxia as has been previously described (18).

The GAD antibodies in those patients with gluten ataxia not on the diet did not significantly change with the exception of 1 patient where the subsequent titre was much higher. Similarly the anti-GAD titre in those patients with idiopathic

Table 3 Patients with positive GAD antibodies (from the SPS, GA, Idiopathic ataxia and IDDM groups) and the spectrum of autoimmune diseases seen. Effect of gluten-free diet on anti-GAD titre in those patients with gluten sensitivity who went onto a gluten-free diet

No.	Disease	Baseline anti-GAD U/ml	Anti-GAD on gluten-free diet U/ml	Anti-GAD not on gluten-free diet U/ml	TD	Vitiligo	IDDM	MG	PA	GS
1	SPS	90	57	105	No	No	Yes	No	No	Yes
2	SPS	98	67	120	No	No	Yes	No	No	Yes
3	SPS	190	89		No	No	Yes	No	No	Yes
4	SPS	80	n/d		Yes	Yes	No	No	No	Yes
5	SPS	4	<0.2		No	No	No	No	No	Yes
6	SPS	190		185	Yes	No	No	Yes	Yes	No
7	Gluten ataxia	3	<0.2		Yes	No	No	No	Yes	Yes
8	Gluten ataxia	4	<0.2		No	No	No	No	No	Yes
9	Gluten ataxia	4	<0.2		Yes	No	No	No	No	Yes
10	Gluten ataxia	38	<0.2		No	No	No	No	No	Yes
11	Gluten ataxia	2	<0.2		No	Yes	Yes	No	No	Yes
12	Gluten ataxia	2	<0.2		No	No	No	No	No	Yes
13	Gluten ataxia	2	<0.2		Yes	No	No	No	No	Yes
14	Gluten ataxia	64	20		Yes	No	No	No	Yes	Yes
15	Gluten ataxia	5	<0.2		No	No	No	No	No	Yes
16	Gluten ataxia	5	<0.2		No	No	No	No	Yes	Yes
17	Gluten ataxia	5	<0.2		Yes	No	No	No	Yes	Yes
18	Gluten ataxia	130		126	No	No	No	No	No	Yes
19	Gluten ataxia	2		2	No	No	No	No	No	Yes
20	Gluten ataxia	3		3	Yes	No	Yes	No	No	Yes
21	Gluten ataxia	126		124	No	No	No	No	Yes	Yes
22	Gluten ataxia	3		35	Yes	No	Yes	No	No	Yes
23	Ataxia idiopathic	108		110	Yes	No	Yes	No	No	No
24	Ataxia idiopathic	7		12	Yes	Yes	No	No	Yes	No
25	Ataxia idiopathic	3		<0.2	Yes	No	No	No	No	No
26	Ataxia idiopathic	20		n/d	No	No	No	No	No	No
27	Ataxia idiopathic	64		55	No	No	No	No	Yes	No
28	Ataxia idiopathic	3		3	No	No	No	No	No	No
29	Ataxia idiopathic	80		80	Yes	No	Yes	No	No	No
30	Ataxia idiopathic	2		2	No	No	No	No	No	No
31	Ataxia idiopathic	4		3	Yes	No	No	No	No	No
32	IDDM only	2.8			No	No	Yes	No	No	No
33	IDDM only	7.7			No	No	Yes	No	No	No
34	IDDM only	13			No	No	Yes	No	No	No
35	IDDM only	20			No	No	Yes	No	No	No
36	IDDM only	3.4			No	No	Yes	No	No	No
37	IDDM only	9.5			No	No	Yes	No	No	No

IDDM, insulin dependent diabetes mellitus; MG, myasthenia gravis; PA, pernicious anaemia; TD, thyroid disease; GS, gluten sensitivity; n/d, not done.

sporadic ataxia did not change significantly on repeated testing with the exception of one patient with low titre at baseline in whom the subsequent test was negative.

Discussion

We have identified a link between gluten sensitivity and GAD antibody associated diseases. Furthermore, we have shown that the titre of GAD antibodies in those patients who also have gluten sensitivity can be influenced by the introduction of a gluten-free diet. The duration of gluten exposure in patients with gluten sensitive enteropathy has been proposed as a risk factor for the development of other autoimmune diseases such as IDDM and hypothyroidism (19). Furthermore, the timing of cereal exposure in infancy has also been shown to increase risk of islet autoimmunity and subsequent development of type 1 diabetes (20). As anti-GAD antibodies appear to be markers of multiple autoimmunity, their reduction/elimination by a gluten-free diet as shown here would support a link between gluten exposure and the development of anti-GAD-associated diseases of which type 1 diabetes and SPS are examples.

The high prevalence of anti-GAD antibodies in our patients with gluten ataxia (40%) blurs the distinction between anti-GAD-associated ataxia and gluten ataxia. Both conditions are immune-mediated and are associated with other autoimmune diseases. Both are characterized by insidious onset of progressive gait ataxia and both have similar age at onset. The presence of oligoclonal bands has been reported in 10 of 14 patients with anti-GAD ataxia and in 12 of 28 patients with

gluten ataxia (11, 21). Furthermore, the HLA DQ2 appears to be overrepresented in both SPS (six of seven patients) and in patients with gluten ataxia (70%). It is very likely that gluten ataxia and anti-GAD-associated ataxia either overlap considerably or are part of the same disease spectrum.

An antibody-mediated pathogenesis in GAD antibody-associated diseases is supported by the observation that serum and CSF samples from patients with SPS contain antibodies capable of selectively suppressing GABAergic transmission (22). Another study has also shown that SPS and anti-GAD-associated ataxia are the direct consequence of antibody-mediated neuronal dysfunction (12). An antibody-mediated pathogenesis is also supported in gluten ataxia, where intraventricular injection of serum from a patient with gluten ataxia has been shown to induce ataxia in mice (23). The only observed difference between these groups of patients with positive anti-GAD was that patients with SPS tend to have a higher mean titre by comparison to patients with gluten ataxia and those with idiopathic sporadic ataxia.

There is no explanation as to the diversity of clinical presentations seen in the context of anti-GAD positivity. Furthermore, not all patients with SPS have circulating GAD antibodies. One or more additional autoimmune disorders are present in 60% of anti-GAD-positive patients with SPS versus 6% in anti-GAD-negative patients (24). A similar observation has recently been reported in patients with IDDM where anti-GAD positive patients have a higher prevalence of autoimmune thyroiditis than anti-GAD negative ones (25). GAD antibodies may therefore simply signify multiple autoimmunity rather than being directly pathogenic. The role of other antibodies including transglutaminase antibodies in this context may merit further consideration, as a possible alternative contributor to the pathogenesis of anti-GAD-associated diseases.

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