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Immune Cross-Reactivity in Celiac Disease: Anti-Gliadin Antibodies Bind to Neuronal Synapsin I[1]

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Celiac disease is an immune-mediated disorder triggered by ingestion of wheat gliadin and related proteins in genetically susceptible individuals. In addition to the characteristic enteropathy, celiac disease is associated with various extraintestinal manifestations, including neurologic complications such as neuropathy, ataxia, seizures, and neurobehavioral changes. The cause of the neurologic manifestations is unknown, but autoimmunity resulting from molecular mimicry between gliadin and nervous system proteins has been proposed to play a role. In this study, we sought to investigate the immune reactivity of the anti-gliadin Ab response toward neural proteins. We characterized the binding of affinity-purified anti-gliadin Abs from immunized animals to brain proteins by one- and two-dimensional gel electrophoresis, immunoblotting, and peptide mass mapping. The major immunoreactive protein was identified as synapsin I. Anti-gliadin Abs from patients with celiac disease also bound to the protein. Such cross-reactivity may provide clues into the pathogenic mechanism of the neurologic deficits that are associated with gluten sensitivity. ***The Journal of Immunology,*** **2007, 178: 6590–6595.**

Celiac disease, or gluten-sensitive enteropathy, is a common autoimmune disorder, with an estimated prevalence of ~1% in many parts of the world (1, 2). It is characterized by inflammation and tissue remodeling in the small intestine. Immune sensitivity to gliadin (a component of gluten) and related cereal proteins is the hallmark of celiac disease, while elimination of the immunogenic proteins from the diet results in the reduction of associated anti-gliadin and anti-transglutaminase Abs, coupled with frequent amelioration of the gastrointestinal symptoms (3). A majority of patients express the HLA-DQ2 and/or -DQ8 MHC class II molecules, which are involved in the presentation of gliadin to T cells (4). Celiac disease is also characterized by systemic manifestations that contribute to a complex clinical presentation. Neurologic deficits, including axonal neuropathy and cerebellar ataxia, are among the most common extraintestinal symptoms associated with celiac disease (5, 6). In addition, elevated levels of anti-gliadin Ab have been associated with idiopathic neuropathy and ataxia, even in the apparent absence of the characteristic mucosal pathology (7–10). The terms gluten ataxia and gluten neuropathy have been proposed to describe these conditions, but the significance of anti-gliadin Abs in the absence of biopsy-proven intestinal damage in this context is a subject of contention (8, 10, 11).

The cause of the neurologic symptoms that are associated with gluten sensitivity remains unknown, although recent findings point to the involvement of immune-mediated mechanisms. Lymphocytic infiltration has been demonstrated in the cerebellum of individuals with ataxia and anti-gliadin Abs, as well as in the peripheral nerve of patients with neuropathy and anti-gliadin Abs (8, 10). In addition, the associated cerebellar ataxia and peripheral neuropathy have been reported to respond in some cases to gluten-free diet or therapy with i.v. Igs (12–14). In other studies, patient serum and anti-gliadin Abs have been shown to bind neural tissue, implying that the Abs may be cross-reacting with autoantigens (15, 16). Based on these findings, a mechanism of molecular mimicry has been postulated for some cases, although identification of a neural autoantigen has remained elusive.

In this study, we investigated the cross-reactivity of animal and human anti-gliadin Abs with nervous system Ags. Purified gliadin-specific Abs from rabbits were found to strongly immunoreact on Western blot analysis with a protein doublet. We identified the cross-reactive doublet as synapsin I, a member of a group of neuronal phosphoproteins involved in the regulation of neurotransmitter release. Ab reactivity to synapsin I was also examined in sera from patients with celiac disease and control subjects by ELISA. Anti-synapsin Ab reactivity was found in several patients with celiac disease, whereas it was absent in control subjects without anti-gliadin Abs.

Materials and Methods

Rabbit and human sera

Animal anti-gliadin Abs were from pooled serum IgG fraction of multiple immunized rabbits (Sigma-Aldrich). Human serum samples were from nine patients with biopsy-proven celiac disease and elevated levels of IgG and/or IgA anti-gliadin Abs, nine patients with neurologic disease without anti-gliadin Abs, and four healthy subjects. Celiac disease patients included three who were also diagnosed with neurologic disease (two with peripheral neuropathy and one with cerebellar ataxia) and six patients without neurologic disease (two of whom also had dermatitis herpetiformis). The

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neurologic disease control group consisted of patients without anti-gliadin Abs, including two with multifocal motor neuropathy, two with chronic inflammatory demyelinating polyneuropathy, two with sporadic cerebellar ataxia, and three with multiple sclerosis. Informed consent was obtained in accordance with protocols approved by the internal review boards of all involved institutions.

Affinity purification of anti-gliadin Abs

Pooled rabbit anti-gliadin Abs or sera from two patients with biopsy-proven celiac disease and high anti-gliadin Ab titer (CD 1 and CD 2) were separately purified by affinity chromatography, using a gliadin-coupled affinity column. The gliadin proteins used for constructing the affinity column were purified from a gliadin preparation (Sigma-Aldrich) by extraction with 70% ethanol. The protein extract was lyophilized and dissolved in 50% *N*,*N*-dimethylformamide at 20 mg/ml. An affinity chromatography column was prepared using the AminoLink-activated agarose gel bead support (Pierce). After packing the column with 4 ml of gel slurry, it was equilibrated with 50% *N*,*N*-dimethylformamide in PBS. This was followed by the addition of 2 ml of the purified gliadin solution and 200 μl of 1 M $NaCNBH_3$ in 10 mM NaOH. The coupling reaction was allowed to continue while gently rotating the column (6 h, room temperature). Remaining reactive sites were blocked by incubation with 1 M Tris (pH 7.4) for 30 min. The gliadin-coupled affinity column was washed and equilibrated with PBS. Purification was initiated by the introduction of Ab solution or serum samples into the column and continuous flow for 1 h. The column was then thoroughly washed with PBS, followed by elution of bound Abs with 100 mM glycine buffer (pH 3.0). The eluted Ab fraction was neutralized with 1 M Tris (pH 7.5). The column flowthrough and the eluted Ab fraction were concentrated by centrifugal filtration.

Binding of anti-gliadin Abs to neural proteins

Mouse brain tissue was homogenized in lysis buffer (20 mM Tris, 5 mM EDTA, and 1% Nonidet P-40, containing a mixture of protease inhibitors (Roche) (pH 7.5)) at 4°C. Homogenized sample was centrifuged at 16,000 × *g* and 4°C for 20 min to remove cellular debris. The interaction of the anti-gliadin Abs with neural proteins was investigated by Western blot analysis. One-dimensional SDS-PAGE (7.5%) or two-dimensional nonequilibrium pH gradient electrophoresis (17) was conducted on 40- to 80-μg protein, followed by transfer onto nitrocellulose membrane. The membrane was blocked with 5% milk in TBS for 2 h. Incubation with affinity-purified anti-gliadin Ab (0.4–7.2 μg/ml) or with the column flowthrough Abs was conducted for 1 h. The HRP-conjugated secondary Ab used was anti-rabbit IgG (Amersham Biosciences), anti-human IgG (Amersham Biosciences), or anti-human IgA (MP Biomedicals). Detection of bound Abs was by the ECL system and autoradiography film. The membrane was reblotted with anti-synapsin I Ab (Chemicon International) after removing bound Abs with a Western blot stripping buffer (Pierce). Once synapsin I was eventually identified as the putative cross-reactive protein (as described in the following sections), the immunoblotting procedure was also done on 0.1 μg of bovine synapsin I (18), deglycosylated bovine synapsin I, BSA (Sigma-Aldrich), and bovine calreticulin (Sigma-Aldrich). The deglycosylated synapsin I was prepared by treatment of synapsin I with trifluoromethanesulfonic acid (TFMS),[3] using a deglycosylation kit according to the manufacturer's protocol (Sigma-Aldrich).

Purification and mass spectrometric identification of protein

The putative target protein was purified by affinity chromatography on an anti-gliadin Ab cross-linked column as follows. Affinity-purified rabbit anti-gliadin Ab from above (500 μg) was added to a 10 mg/ml solution of sodium *m*-periodate and incubated in the dark for 30 min. The oxidized Ab was desalted and added to a CarboLink column (Pierce), where the newly formed aldehyde groups on the Fc portion of the Ab reacted with the hydrazide groups on the matrix. The reaction was allowed to proceed overnight at 4°C, after which the column was drained and washed with TBS. Affinity separation was conducted on 4 mg of mouse brain lysate protein. Affinity column-bound Ag was eluted with 100 mM glycine buffer (pH 3.0). Eluted protein fractions were monitored at 280 nm and neutralized with 1 M Tris. Pooled protein fractions of interest were precipitated by the chloroform-methanol method (19) and analyzed by gel electrophoresis and Coomassie staining.

The protein band of interest at ~75 kDa was excised from the gel, washed, and destained. It was then reduced by adding 100 μl of 0.01 M DTT in 0.1 M Tris (pH 8.5) and heating (55°C, 1–2 h). After cooling, the liquid was replaced with 100 μl of 0.015 M iodoacetamide in 0.1 M Tris (pH 8.5). The reaction was allowed to proceed for 30 min in the dark, after which the liquid was removed. The gel was prepared for digestion by washing once with 200 μl of 0.05 M Tris (pH 8.5)/25% acetonitrile and twice with 200 μl of 0.05 M Tris (pH 8.5)/50% acetonitrile for 20 min. After removing the washes, the gel was dried for 30 min and digested by adding 0.08 μg of trypsin (Roche) in 25 μl of 0.025 M Tris (pH 8.5) and incubating in a heating block (32°C, 12 h). Peptides were extracted with 50% acetonitrile/2% trifluoroacetic acid. The combined extracts were reduced in volume to ~15 μl and transferred to an injection vial. Liquid chromatography tandem mass spectrometry (MS/MS) analysis was done on a Micromass Q-Tof hybrid quadrupole/time-of-flight mass spectrometer with a nanoelectrospray source (Waters). Capillary voltage was set at 1.8 kV and cone voltage at 32V; collision energy was set according to mass and charge of the ion from 14 to 50 eV. Chromatography was performed on a LC Packings HPLC system (Dionex) with a C18 PepMap column (Dionex) using a linear acetonitrile gradient at 200 nl/min. Raw data files were processed using the MassLynx ProteinLynx software (Waters), and .pkl files were subjected to a search, using the Mascot program at www.matrixscience.com.

Ab measurement in human sera

Anti-gliadin and anti-synapsin I Ab reactivities were analyzed by ELISA. Wells in 96-well round-bottom polystyrene microtiter plates (BD Biosciences) were covered with 50 μl of a 10 μg/ml solution of the gliadin extract or a 3 μg/ml solution of purified bovine synapsin I in 0.1 M carbonate buffer (pH 9.6). Coating buffer alone was added to control wells. After overnight incubation at 4°C, wells were washed and blocked by incubation with 1% BSA in PBS containing 0.05% Tween 20 for 1.5 h at room temperature. Serum samples were diluted at 1/400 (for gliadin-coated wells) or at 1/200 (for synapsin-coated wells) and added at 50 μl/well in duplicates. After washing the wells as before, 50 μl of peroxidase-conjugated goat anti-human IgG (Amersham Biosciences) or IgA (MP Biomedicals) secondary Ab (1/1600 dilution) was added to each well, and the plates were incubated for 1 h. The wells were washed, and 50 μl of developing solution, comprising 27 mM citric acid, 50 mM Na_2HPO_4, 5.5 mM *o*-phenylenediamine, and 0.01% H_2O_2 (pH 5), was added to each well. After incubating the plates at room temperature for 30 min, absorbance was measured at 450 nm. Absorbance values were corrected for nonspecific binding by subtraction of the mean absorbance of the associated control wells.

Results

The purified gliadin extract used for preparation of the affinity column contained proteins in the 25- to 50-kDa range (Fig. 1*A*). We first examined the binding of the affinity-purified anti-gliadin Abs (from pooled serum of gliadin-immunized rabbits) to mouse brain proteins by one- and two-dimensional gel electrophoresis and immunoblotting. The purified Abs strongly bound to two closely spaced bands with molecular masses of 73–78 kDa and isoelectric points of 9.5–10.5 (Fig. 1, *B*, *C*, and *E*). The column flowthrough Abs (from which the anti-gliadin Abs had been removed) did not bind to these bands (Fig. 1*D*).

To further characterize the putative immunoreactive protein, it was purified from mouse brain tissue by affinity chromatography, using an anti-gliadin Ab cross-linked column. The SDS-PAGE profile of eluted fractions showed a band at ~75 kDa (data not shown). The excised protein was digested with trypsin after chemical modification and analyzed by liquid chromatography-MS/MS (Fig. 2). The detected peptide masses were searched against the National Center for Biotechnology Information (NCBI) protein sequence database. Results of the database search revealed synapsin Ib (accession no. AAD09833) as a significant candidate protein, with seven peptides identified and 17% sequence coverage (Fig. 2) (20). Two-dimensional immunoblotting of brain proteins using anti-gliadin Ab and reblotting with anti-synapsin I Ab clearly demonstrated binding to the same two bands, representing synapsin Ia and synapsin Ib (Fig. 1*F*). The two-dimensional profile of the proteins also agreed with that previously reported for synapsin I (21).

[3] Abbreviations used in this paper: TFMS, trifluoromethanesulfonic acid; MS/MS, tandem mass spectrometry.

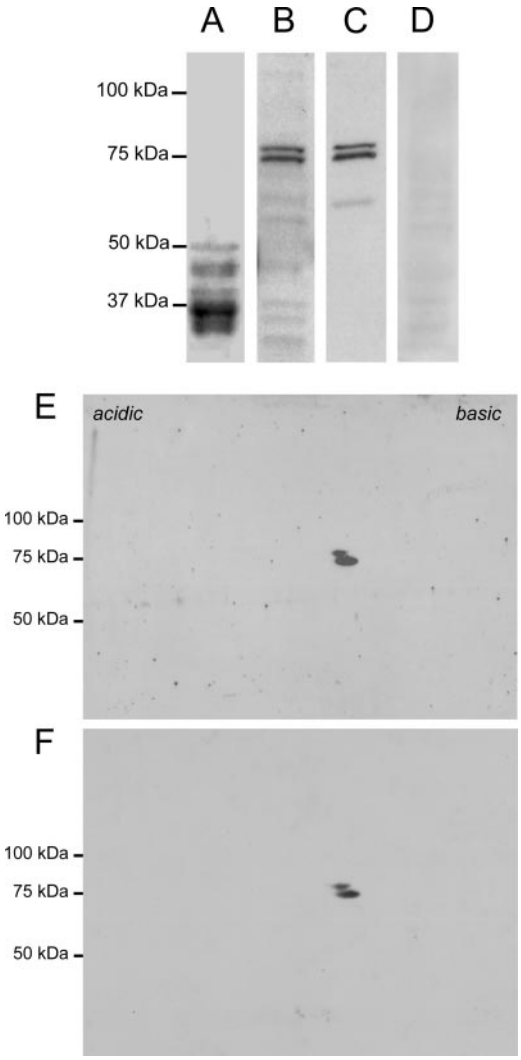
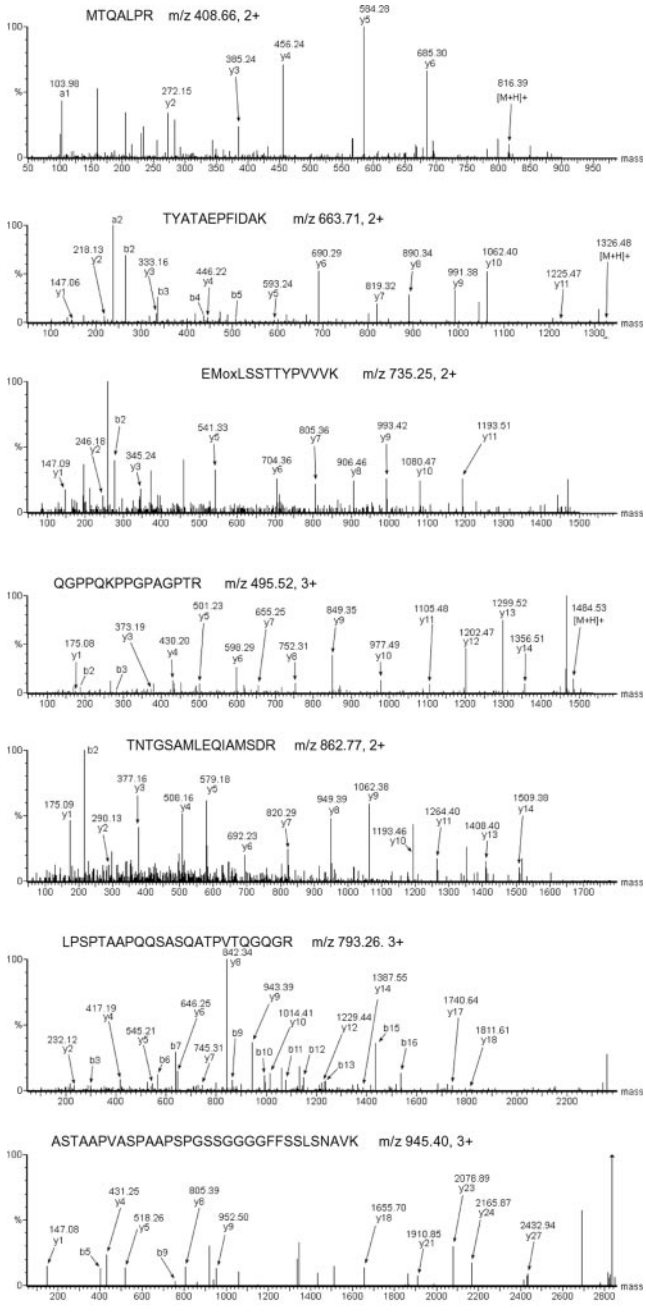


FIGURE 1. Immunoreactivity of anti-gliadin Ab in nervous system tissue. *A*, SDS-PAGE profile of extracted gliadin (15 μg) used for constructing the affinity column, following transfer to nitrocellulose membrane and staining with Ponceau S. *B* and *C*, One-dimensional immunoblots of mouse brain lysate with commercial IgG fraction anti-gliadin Ab (*B*), affinity-purified anti-gliadin Ab preparation (*C*), or affinity column flowthrough fraction from which anti-gliadin Abs had been removed (*D*). *E*, Two-dimensional immunoblot of mouse brain lysate with affinity-purified anti-gliadin Ab. *F*, Reblotting of nitrocellulose membrane from *D* with anti-synapsin I Ab shows binding to the same two bands, representing synapsin Ia (*upper band*) and synapsin Ib (*lower band*).

Binding of affinity-purified anti-gliadin Ab from gliadin-immunized rabbits to purified synapsin Ia/Ib and to none of the controls by Western blot analysis further confirmed the identity of the protein bands (Fig. 3*A*).

To determine whether human anti-gliadin Abs also bind to synapsin I, we affinity purified serum anti-gliadin Ab from two patients with biopsy-proven celiac disease and elevated anti-gliadin Abs. Immunoblotting clearly demonstrated the binding of patient gliadin-specific IgG and IgA Abs to synapsin I, whereas no binding to BSA and bovine calreticulin was detected (Fig. 3, *B* and *C*). On the basis of these results, we established an ELISA for the detection of anti-synapsin I Abs in serum. Serum samples were either from subjects with clearly elevated anti-gliadin Ab titers or from controls without anti-gliadin Abs, either with or without neurologic symptoms. Five of nine patients with gluten sensitivity (three of three with and two of five without neurologic disease)

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  1 MNYLRRRLSD SNFMANLPNG YMTDLQRPQP PPPPPSAASP GATPGSATAS
 51 AERASTAAPV ASPAAPSPGS SGGGGFFSSL SNAVKQTTAA AAATFSEQVG
101 GGSGGAGRGG AAARVLLVID EPHTDWAKYF KGKKIHGEID IKVEQAEFSD
151 LNLVAHANGG FSVDMEVLRN GVKVVRSLKP DFVLIRQHAF SMARNGDYRS
201 LVIGLQYAGI PSVNSLHSVY NFCDKPWVFA QMVRLHKKLG TEEFPLIDQT
251 FYPNHKEMLS STTYPVVVKM GHAHSGMGKV KVDNQHDFQD IASVVALTKT
301 YATAEPFIDA KYDVRVQKIG QNYKAYMRTS VSGNWKTNTG SAMLEQIAMS
351 DRYKLWVDTC SEIFGGLDIC AVEALHGKDG RDHIIEVVGS SMPLIGDHQD
401 EDKQLIVELV VNKMTQALPR QPQRDASPGR GSHSQSSSPG ALTLGRQTSQ
451 QPAGPPAQQR PPPQGGPPQP GPGPQRQGPP LQQRPPPQGQ QHLSGLGPPA
501 GSPLPQRLPS PTAAPQQSAS QATPVTQGQG RQSRPVAGGP GAPPAARPPA
551 SPSPQRQAGA PQATRQASIS GPAPTKASGA PPGGQQRQGP PQKPPGPAGP
601 TRQASQAGPG PRTGPPTTQQ PRPSGPGPAG RPAKPQLAQK PSQDVPPPIT
651 AAAGGPPHPQ LKASPSQAQP
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FIGURE 2. MS/MS spectra of tryptic peptides matching synapsin Ib. Sequence of the peptide is given at the *top* of each spectrum along with the *m/z* value and charge state of the parent ion. For individual fragment ion spectra, y ions resulting from cleavage at the peptide amide bond (40) are indicated by arrows and the *m/z* values given. All fragment ions in the spectra have a +1 charge state. *X*-axis, *m/z*; *y*-axis, relative ion intensity.

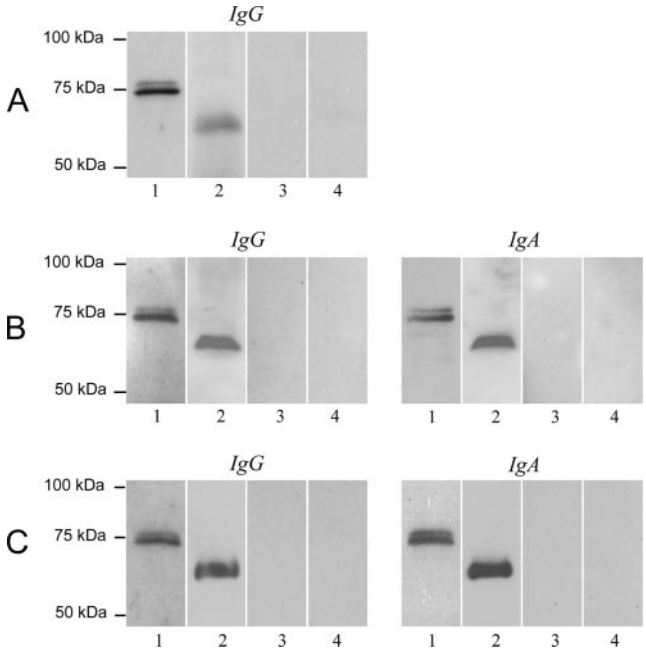

FIGURE 3. Binding of anti-gliadin Ab to synapsin I, deglycosylated synapsin I, and control proteins. *A–C*, Immunoblotting of purified bovine synapsin I (*lane 1*), deglycosylated (TFMS-treated) bovine synapsin I (*lane 2*), BSA (*lane 3*), and bovine calreticulin (*lane 4*) with affinity-purified rabbit IgG anti-gliadin Ab (*A*), and affinity-purified IgG and IgA anti-gliadin Ab from two celiac disease patients, CD 1 (*B*) and CD 2 (*C*).

exhibited elevated levels of IgG and/or IgA anti-synapsin I Abs (Table I). None of the control sera without gluten sensitivity showed significant Ab reactivity to synapsin I.

To examine the possible role of the synapsin I carbohydrate groups in the Ab interaction, the immunoblotting procedure was repeated with the affinity-purified rabbit and human anti-gliadin Abs after treatment of synapsin I with TFMS. The results showed that, while the protein was deglycosylated, with a molecular mass shift to ~60–65 kDa, the binding to anti-gliadin Abs was not inhibited (Fig. 3).

Discussion

The objective of this study was to examine the cross-reactivity of the anti-gliadin humoral immune response in neural tissue. We found that both animal and human anti-gliadin Abs can cross-react with synapsin I, a cytosolic phosphoprotein found in most neurons of the central and peripheral nervous systems (22, 23). The anti-gliadin Abs bound to both isomers of synapsin I, a and b, which have very similar amino acid sequences. The b isomers in human and mouse differ from the a isomers by deletions and sequence polymorphisms at the carboxyl terminus. Additionally, mouse synapsin Ib has a unique deletion of aa 571–598. Apart from these, the b isomers are identical in sequence to their corresponding a isomers.

In examining the amino acid sequences of gliadins available in the NCBI database, it is apparent that a typical homology search (such as basic local alignment search tool) would not return a significant hit to synapsin I. However, the similarity between synapsin I and gliadin derives from having regions with high frequencies of proline and glutamine residues. In synapsin, the proline/glutamine rich region is in the carboxyl half of the protein. In gliadins, the multiple prolines and glutamines (including several poly-Q regions) are distributed throughout the sequence. More specifically, gliadins have several PQP and PQQP motifs, which are also found in synapsin I. The presence of these amino acid sequences may be partly responsible for the observed cross-reactivity of the anti-gliadin Abs. In contrast, while synapsin I is

Table I. *Anti-gliadin and anti-synapsin I Ab reactivities in human sera, as determined by ELISA*[a]

	IgA		IgG	
Serum Sample	Anti-gliadin Ab[b]	Anti-synapsin I Ab[b]	Anti-gliadin Ab[b]	Anti-synapsin I Ab[b]
CD 1[c]	0.871 ± 0.031	0.477 ± 0.014	1.205 ± 0.012	0.564 ± 0.012
CD 2[c]	1.430 ± 0.062	0.811 ± 0.020	0.538 ± 0.019	0.606 ± 0.045
CD 3[d]	0.128 ± 0.008	0.115 ± 0.005	1.250 ± 0.008	0.332 ± 0.009
CD 4	0.035 ± 0.008	0.011 ± 0.006	1.021 ± 0.081	0.021 ± 0.007
CD 5	0.070 ± 0.006	0.028 ± 0.004	1.338 ± 0.133	0.046 ± 0.010
CD 6	0.055 ± 0.010	0.013 ± 0.008	1.024 ± 0.102	0.032 ± 0.017
CD 7	0.499 ± 0.014	0.010 ± 0.004	1.521 ± 0.017	0.327 ± 0.007
CD 8[e]	0.325 ± 0.020	0.116 ± 0.006	0.875 ± 0.036	0.059 ± 0.010
CD 9[e]	0.020 ± 0.004	0.021 ± 0.009	0.266 ± 0.022	0.022 ± 0.013
PN 1 (MMN)	0.012 ± 0.002	0.038 ± 0.008	0.014 ± 0.010	0.025 ± 0.019
PN 2 (MMN)	0.022 ± 0.002	0.028 ± 0.007	0.038 ± 0.006	0.048 ± 0.006
PN 3 (CIDP)	0.006 ± 0.004	0.006 ± 0.006	0.014 ± 0.002	0.008 ± 0.009
PN 4 (CIDP)	0.013 ± 0.003	0.029 ± 0.007	0.068 ± 0.003	0.011 ± 0.007
CA 1	0.014 ± 0.006	0.014 ± 0.001	0.047 ± 0.004	0.019 ± 0.011
CA 2	0.019 ± 0.004	0.036 ± 0.008	0.037 ± 0.005	0.042 ± 0.012
MS 1	0.028 ± 0.008	0.020 ± 0.009	0.039 ± 0.002	0.019 ± 0.012
MS 2	0.019 ± 0.006	0.037 ± 0.005	0.031 ± 0.011	0.042 ± 0.017
MS 3	0.011 ± 0.006	0.008 ± 0.003	0.042 ± 0.013	0.030 ± 0.016
Healthy control 1	0.011 ± 0.004	0.006 ± 0.008	0.057 ± 0.002	0.040 ± 0.017
Healthy control 2	0.005 ± 0.012	0.002 ± 0.002	0.077 ± 0.008	0.027 ± 0.012
Healthy control 3	0.016 ± 0.007	0.009 ± 0.007	0.038 ± 0.004	0.030 ± 0.008
Healthy control 4	0.006 ± 0.006	0.025 ± 0.005	0.043 ± 0.005	0.027 ± 0.011

[a] CD, celiac disease; PN, peripheral neuropathy; CA, cerebellar ataxia; MMN, multifocal motor neuropathy; CIDP, chronic inflammatory-demyelinating polyneuropathy; MS, multiple sclerosis.
[b] Values represent the mean for the difference between optical densities of Ag-coated and control wells ± SD.
[c] Celiac disease patients CD 1 and CD 2 were also diagnosed with peripheral neuropathy.
[d] Celiac disease patient CD 3 was also diagnosed with sporadic cerebellar ataxia.
[e] Celiac disease patients CD 8 and CD 9 also had dermatitis herpetiformis.

known to carry O-linked *N*-acetylglucosamine and fucosyl groups (24, 25), the removal of these carbohydrates do not inhibit the binding of anti-gliadin Abs to the protein, ruling out a major role for them as epitopes in the Ab-Ag interaction.

In the human serum samples, Ab to synapsin I was detected in several patients with gluten sensitivity, while control specimens without anti-gliadin Ab did not exhibit significant anti-synapsin Ab reactivity. The patient data also clearly demonstrated that anti-gliadin Ab levels do not necessarily correlate with anti-synapsin Ab reactivity and that only certain subsets of anti-gliadin Abs cross-react with synapsin I. Because of the large number and heterogeneous nature of gliadins, as well as the high diversity of wheat phenotypes (26), the anti-gliadin immune response is likely to involve a sizeable repertoire of antigenic determinants. Therefore, varying degrees of cross-reactivity to synapsin I can be expected in different patients with gluten sensitivity. Such differences in the anti-gliadin Ab cross-reactivity in different patients may reveal clues about the potential pathogenic role of the Ab and its association with specific extra-intestinal complications.

Whether Ab cross-reactivity to synapsin I can exert a pathogenic effect in gluten sensitivity is unknown. Synapsins appear to be multifunctional proteins, containing different domains that confer specific activities (27). In addition to binding synaptic vesicles and various cytoskeletal proteins (18, 28, 29), synapsins share structural similarity in the C domain with a group of ATP-dependent synthases, suggesting a possible enzymatic function (30, 31). Disruption of synapsin activity in a number of experiments using gene deletion, anti-synapsin Ab, or neutralizing peptides indicates that synapsin is required in forming and sustaining the reserve pool of synaptic vesicles and thereby regulating neurotransmitter release (27, 32, 33). Specifically, binding of anti-synapsin I Abs to the aplysia synapsin homolog has been shown to reduce posttetanic potentiation and to increase the rate and extent of synaptic depression (33).

Although pathogenic Abs typically target Ags in the extracellular matrix or on the cell surface, there is evidence that Abs to intracellular Ags can also cause disease. For example, passive transfer of Abs to amphiphysin, a protein that is associated with the cytoplasmic surface of synaptic vesicles, has been shown to reproduce the stiff-person syndrome in experimental animals (34). In addition, Abs to glutamic acid decarboxylase, which catalyzes the production of the neurotransmitter γ-aminobutyric acid, have been shown to selectively suppress γ-aminobutyric acid-mediated synaptic transmission (35). As synapsin I is associated with synaptic vesicles, it might be similarly targeted by Abs taken up from the extracellular compartment. Therefore, it is conceivable that, in some patients with gluten sensitivity, the anti-gliadin Ab response would affect synapsin I activity, thus interfering with neurotransmitter release and resulting in neurologic dysfunction. The immune cross-reactivity may also lead to tissue damage through T cell-mediated mechanisms. Among the celiac patients in this study, anti-synapsin Abs were present in subjects with neurologic disease, as well as those without. This implies that, like other autoimmune disorders, Ab reactivity is only one piece of the puzzle in the pathogenic mechanism of the neurologic complications of celiac disease. Therefore, the potential pathogenic role of antisynapsin immune cross-reactivity in the neuropathy or CNS manifestations is likely to depend on a number of additional factors, including the type and fine specificity of the immune response, local integrity of the blood-nerve or blood-brain barrier, and presence of proinflammatory factors. Similar features affect the central and peripheral neurologic syndromes associated with paraneoplastic Abs (36).

Finally, it should be noted that the pathogenic effect of antisynapsin immune reactivity might not be limited to the nervous system. As the presence of synapsin I has been demonstrated recently in nonneuronal cells (although at low levels), including liver epithelial cells (37) and pancreatic β cells (38), Ab cross-reactivity to synapsin I may be affiliated with other extraintestinal manifestations of gluten sensitivity as well. Ab and T cell access to these tissues would be more easily facilitated than to the nervous system. In addition, our study does not rule out the possibility of anti-gliadin Ab cross-reactivity toward other autoantigens. In another study, for example, anti-gliadin Abs were reported to cross-react with calreticulin (39), although we did not detect such cross-reactivity using affinity-purified rabbit and human anti-gliadin Abs.

In this study, we have shown that the anti-gliadin Ab response in immunized animals and celiac patients can cross-react with the synapsin I protein. Although the results are preliminary and should not be interpreted as indicating an association with neurologic pathogenicity, the identification of a potential neuronal autoantigen in celiac disease offers new opportunities for investigating the poorly understood connection between gluten sensitivity and neurologic deficits. To better understand the described Ab reactivity and determine whether it is associated with, or has any role in, the extraintestinal complications of celiac disease, further work involving longitudinal studies in patients and experimental animals will be undertaken.

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Disclosures

The authors have no financial conflict of interest.

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