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Specificity Analysis of Anti-gliadin Mouse Monoclonal Antibodies Used for Detection of Gliadin in Food for Gluten-free Diet

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A gluten-free diet (GFD) is the sole effective long-lasting treatment of celiac disease. Four monoclonal antibodies (Abs) were prepared by immunization of animals kept on GFD with gliadin. The specificity of these Abs to decapeptides of α- and γ-gliadin and ω-secalin was analyzed by the PEPSCAN technique. Repetitive sequences of α- and γ-gliadin and ω-secalin containing the motifs QPFP**X**Q (**X** = Q, L, P) were recognized by all Abs tested. These Abs also frequently reacted with peptides containing the sequences QQSFPQQ, QQTFPQP, and QPFRPQ. On the basis of PEPSCAN results two Abs—8D4 and 7C6—were selected for the construction of a new ELISA kit for the detection of gliadin in food. The comparison of data obtained using the newly developed ELISA kit and commercially available ones indicated that Abs selection on the basis of their fine specificity to gliadin is useful for sensitive detection of gliadin in foods.

KEYWORDS: Antibody specificity; gliadin; celiac disease

INTRODUCTION

Celiac disease (CLD) is induced by dietary gluten (the major storage protein in wheat kernels) in genetically susceptible individuals. Ingestion of gluten leads to severe damage to small gut mucosa characterized by lymphocytic infiltration, inflammation, villous flattening, and crypt hyperplasia. Active CLD is manifested by characteristic gastrointestinal symptoms such as diarrhea, abdominal pain, and weight loss; however, atypical and silent forms of CLD occur frequently in adults. The most severe complication of CLD in patients who do not adhere to a gluten-free diet (GFD) is the development of gastrointestinal malignancies—lymphoma and carcinoma. Omitting gluten from the diet of CLD patients leads to the full recovery of small intestine architecture and functions and the disappearance of clinical symptoms. Gluten-containing food is able to exacerbate in patients on GFD the disease with all of the above-mentioned clinical and immunopathological consequences (*1*−*4*).

Wheat gluten is a mixture of various polypeptides, which can be divided into alcohol-soluble gliadins and alcohol-insoluble glutenins. Electrophoresis at acidic pH reveals five major gliadin subfractions, α, β, γ, δ, and ω, of molecular weight ranging from 30000 to 75000 and consisting of 250−650 amino acids. Gliadin and related proteins in other cereals, that is, secalins (rye), hordeins (barley), and avenins (oat), are rich in proline and glutamine and belong to the group of prolamins. The capability to induce CLD decreases from gliadins to avenins (*5*).

The availability of gluten-free or low-gluten (below 4 mg of gluten/100 g of food, 40 ppm) foodstuffs is crucial for the quality of CLD patients' lives. However, commercial food products declared as gluten free can be relatively often contaminated by gluten [in the range of 20−200 ppm (mg/kg)] (*6*). Moreover, gluten is also unexpectedly present in many food products, such as marmalades, mayonnaise, beer, and sausages. Ingestion of gluten-containing food evokes in CLD patients on GFD infiltration of lamina propria by leukocytes, increases the number of intraepithelial lymphocytes and their stimulation, and induces remodeling small intestinal mucosa toward hyperplasia of crypts and villous atrophy. Permanent irritation of gut mucosa of CLD patients can subsequently lead to resistance to GFD—refractory sprue and development of gastrointestinal malignant diseases (*7*−*9*). Detection of gliadin and related plant proteins was performed by various methods of instrumental analysis, such as chromatography, sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE), and mass spectrometry as well as immunologically based enzyme-linked immunosorbent assay (ELISA) technique (*10*−*13*). The results of commercially

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available ELISAs for detection of gliadins in food vary, reflecting differences in extraction procedure and the specificity of anti-gliadin antibodies (Abs) used for this assay (*14*).

The aim of our study was to prepare and characterize in detail mouse monoclonal anti-gliadin Abs for the purpose of a specific and sensitive ELISA kit detecting gliadins and related prolamins in food products. The PEPSCAN technique with sets of overlapping decapeptides covering the whole sequence of α-gliadin, γ-gliadin, and ω-secalin was used to characterize antigenic epitopes recognized by Abs. The efficiency of the newly developed ELISA constructed on the basis of Abs selected after determination of their fine specificity to gliadin was verified in a comparative pilot study with other commercially available kits.

MATERIALS AND METHODS

Preparation of Mouse Monoclonal Anti-gliadin Antibodies and Their Detection by ELISA. Balb/c mice, kept on a gluten-free diet for two generations, were injected with two doses of 50−100 μg of crude gliadin (Sigma) in CFA sc and a boosting dose iv without CFA. The levels of serum IgG anti-gliadin Abs were repeatedly tested by direct, two-step ELISA. The ELISA was done according to a procedure described for testing serum levels of human anti-gliadin Abs (*15*). Briefly, crude gliadin (Sigma) was first dissolved in 70% ethanol and then diluted in phosphate-buffered solution (PBS) at a final concentration of 10 μg/mL. Each well of a 96-well microtiter plate (Gama) received 100 μL of this solution. After incubation at 4 °C overnight, the absorbed gliadins were fixed with 0.25% glutaraldehyde solution (10 min). Subsequently, the wells were washed by PBS and PBS−Tween (0.05%) and blocked by 1% bovine serum albumin, in PBS for 1 h at room temperature (RT). The sera, supernatants from hybridoma cultures diluted in blocking solution (1/50, 1/100, 1/500), or isolated anti-gliadin Abs at a concentration of 0.5, 0.3, or 0.1 mg/mL were applied into plates in triplicate for 2 h at RT. Horseradish peroxidase labeled anti-mouse IgG1 Abs (The Binding Site) diluted (1:1000) in blocking solution were added for 1 h at RT after the washing step. The enzyme reaction was developed by adding a solution containing H_2O_2 and 1,2-*o*-phenylenediamine and stopped by 2 M H_2SO_4. The optical density was read on Titertek Multiscan MCC/340 spectrophotometer (Flow Lab., Irvine, Scotland) at 492 nm.

The cell fusion, selection of Abs producing hybridomas, and ascitic fluid preparation were performed as described previously (*16*). In brief, spleen was dissected from the mouse and passed through the nylon filter to obtain a suspension of splenocytes. After several washings (1200 rpm, 5 min) with Iscove's Modified Dulbecco's Medium with 25 mM Hepes and L-glutamine (IMDM) (Cambrex), the cells were mixed with a suspension of myeloma cells SP20 in a 5:1 ratio. The suspension of both cells was centrifuged in a Falcon-50 mL tube, and the supernatant was aspirated. The tube with the pellet of cells was placed in a 37 °C water bath, and slowly, drop by drop, 1 mL of prewarmed (37 °C) polyethylene glycol (Roche) was added to the cells. The cells were continuously stirred with the pipet tip. Following this step, 1 mL of prewarmed IMDM was repeatedly added to the cells. Finally, 10 mL of the medium was added dropwise to the cell suspension. Subsequently, the cell suspension was centrifuged, and the supernatant was aspirated, resuspended in hybridoma medium [IMDM−fetal calf serum (10% v/v, Cambrex), hypoxanthine−thymidine (1% v/v, Sigma), nonessential amino acids (5% v/v, Sevac), Il-6 (100 units/mL, Pierce], and distributed into 96-well microtiter plates. The day after fusion, hybridoma medium enriched by aminopterine (1% v/v, Sigma) was added (50 μL/well) to each well. The supernatants from wells containing the clone were repeatedly tested for the presence of IgG anti-gliadin Abs by ELISA.

We analyzed the cross-reactivity of mouse monoclonal anti-gliadin Abs with various cereal grains (purchased from collections of the Agriculture University in Prague) and food components: chicken egg albumin (Sigma), soybean flour (Sigma), casein (Sigma), and gelatin (Sigma) using an ELISA test. Grains were converted to powder, extracted by ethanol, and diluted in PBS similarly to the procedure used for crude gliadin. Chicken egg albumin, soybean flour, casein, and gelatin were diluted in PBS. The protocol for the ELISA test was identical with that we used for the detection of gliadin in food. We found cross-reaction of these antibodies with ethanol-extracted cereals: wheat (100%), spelt (100%), rye (100%), barley (20%). No cross-reactivity was found with buckwheat, maize, oat, rice, chicken egg albumin, casein, gelatin, and soybean flour. This procedure was used to ensure the specificity of the gliadin-detecting ELISA kit by excluding anti-gliadin Abs cross-reacting with the above-mentioned food components. Selected clones were transplanted in the peritoneum of syngenic mice. The reactivity of 8D4, 7C6, 5C7, and 6H5 monoclonal anti-gliadin Abs present in ascitic fluid was evaluated by ELISA using crude gliadin as an antigen (**Table 1**). The highest reactivity was detected for Abs 6H5 and 8D4.

Table 1. Dilution of Mouse Monoclonal Anti-gliadin Antibodies Evaluated by ELISA

dilution of antibodies	optical density			
	8D4	7C6	5C7	6H5
10^6	2.85	2.46	2.0	3.1
5×10^6	2.24	1.75	1.42	2.31
10^7	1.52	0.72	0.58	1.53
5×10^7	0.91	0.49	0.47	0.96
10^8	0.38	0.25	0.38	0.42

[a] Antigen, crude gliadin.

Affinity chromatography was used for isolating IgG1 isotype of monoclonal Abs. A 1 mL MAbTrap G II Kit protein G Sepharose high-performance column (Supelco, Bellefonte, PA) was attached to a high-performance liquid chromatography (HPLC) system with a programmable multiwavelength detector set at 280 nm. The system was equilibrated with 20 mM phosphate buffer (3.8 mM Na_2HPO_4, 16.2 mM Na_2HPO_4, pH 7.4). After ascitic fluid (0.2 μm filtered and adjusted to pH 7.4) had been loaded onto the column, bound IgG was eluted with 5 mL of 0.1 M citric acid, pH 2.6. The eluates were immediately neutralized with 100 μL of 1 M Tris-base (Serva). The purity of the Abs preparations was determined by SDS-PAGE (10% gel), followed by Coomassie blue staining. Isolated mouse monoclonal Abs (6H5, 5C7, 8D4, and 7C6) were conjugated with horseradish peroxidase via an Ez-Link Activated Peroxidase Antibody Labeling Kit (Pierce) following the recommendation of the manufacturer and used in PEPSCAN experiments.

PEPSCAN Analysis of Monoclonal Antibodies. Four hundred and seven decapeptides covering the whole sequence of α-gliadin (peptides 1−146), γ-gliadin (181−318), and ω-secalin (391−555) on cellulose membranes were prepared by automated spot synthesis (*17*) as described in detail (*18*). The peptides were covalently bound to a cellulose membrane via their C termini. PEPSCAN membranes were purchased from Abimed. Gliadins and secalin peptides overlapped each other by eight amino acid residues. The amino acid sequences of gliadins and secalin were based on published data (*19*−*21*). PEPSCAN analysis was performed according to the modified method of Osman et al. (*22*): after washing in methanol and TBS-T buffer (NaCl, 137.0 mM; KCl, 2.7 mM; Tris, 50.4 mM; Tween, 0.1%, pH 8) the membranes were blocked in TBS-T containing 5% low-fat milk, washed in TBS-T, and incubated with peroxidase-conjugated mouse monoclonal anti-gliadin Abs diluted in blocking solution at a final concentration of 0.3 mg of immunoglobulin/mL. PEPSCAN membrane was incubated with 20 mL of diluted Abs. After further washings, chemiluminescence was developed using ECL reagents (Amersham), and light emission was detected in various periods using MXB film (Kodak).

The binding intensity of monoclonal anti-gliadin Abs to individual prolamin peptides was assessed semiquantitatively on the basis of spot density and diameter. The data were digitalized, and image analysis was performed using a luminescence detector (Las 1000, Fujifilm). The binding intensity was expressed as the intensity of reactivity (IR) and evaluated in three degrees on an internal scale: strong (3), middle (2), and weak (1). The specificity of each Abs by PEPSAN was analyzed in, at least, three independent experiments. Presented IR are medians of obtained values.

Preparation of Food Samples. We analyzed 19 commercially available foodstuffs declared as gluten-free, for example, various kinds of gluten-free cakes, a sponge biscuit, bread crumbs, buckwheat flour (Jizerské pekárny, sro), a powdered mixture of buckwheat omelette, husked millet, a naturally gluten-free mixture for breadmaking (Labeta, sro), a naturally gluten-free mixture for making dumplings, and a naturally gluten-free mixture for making pancakes (Paleta, sro). These foodstuffs were converted to powder consistency using a Pulverisette 14 rotor-speed mill (Fritsch GmbH, Manufacturers of Laboratory Instruments, Idar-Oberstein, Germany). The samples were mixed carefully in glass flasks. Afterward, foodstuffs were extracted according to the instructions of the manufacturer, in brief: 1 g of sample was transferred to 10 mL of 50% (v/v) ethanol for 30 min and sonicated at RT. After centrifugation (1800*g*, 15 min), the supernatant was diluted 1/500 in diluting buffer (Riedel-de Haën, GmbH, 45213). In the case of Immunotech, a Beckman Coulter Co., and Ridascreen Gluten, article R6101, protocols, 1 g of sample was extracted in 40% (v/v) ethanol and vortexed for 60 s. Supernatants were diluted 1/100 (Immunotech) and 1/20 Ridascreen Gluten) prior to use.

ELISA Tests for the Detection of Gliadin in Food. All tested assays for the detection of gliadin in food were direct two-step sandwich ELISAs in the format of a microtiter plate with 96 wells. Immunotech's ELISA kit employed rabbit polyclonal anti-gliadin Abs (capture Abs) and a combination of two anti-gliadin mouse monoclonal horseradish peroxidase conjugated Abs as detection Abs. ELISA tests for the detection of gliadin were performed according to the instruction of the manufacturers (Ridascreen Gluten, article R6101, Riedel-de Haën, GmbH, [45213]; gliadin ELISA kit, IM3717, Immunotech). Samples were analyzed in three independent experiments, and the average value was considered. The results of ELISA assays were expressed as weight of prolamin per kilogram of foodstuff. Samples containing >100 mg of prolamins/kg were considered as positive and those containing prolamin below 100 mg/kg, negative.

RESULTS AND DISCUSSION

Antibody Specificity. We analyzed and compared the specificity of mouse Abs prepared by immunization of mice kept on GFD with crude gliadin. To analyze antibody specificity we used the PEPSCAN technique with decapeptides covering the whole sequences of α- and γ-gliadin and ω-secalin. Reactivity of mouse Abs 6H5, 5C7, 8D4, and 7C6 with decapeptides of prolamins is shown in **Figure 1**. As documented, these Abs recognize a number of antigenic epitopes on α- and γ-gladin and ω-secalin molecules. The highest number of antigenic epitopes was recognized on ω-secalin, fewer on γ-gliadin, and the smallest number on α-gliadin. The antigenic epitopes recognized by Abs spanned almost the whole sequence of ω-secalin, wheres the reactivity with α- and γ-gliadin-derived peptides was strictly directed to epitopes localized at the N-terminal half of the molecules. The broad spectrum of epitopes could be explained by repetitive sequences and similarity of primary sequences of immunogenic epitopes of prolamins.

We found differences in the specificity and binding intensity of the four anti-gliadin Abs tested. Antibody 5C7 reacted with a number of peptides corresponding to ω-secalin and γ-gliadin and only with one peptide of α-gliadin. Antibody 6H5 reacted with a spectrum of ω-secalin peptides similar to that of Abs 5C7, however, with lower intensity than these Abs. Antibody 6H5 recognized few sequences of γ-gliadin, but mainly with high intensity. These Abs reacted with several peptides in the N-terminal part of the α-gliadin molecule and, interestingly, also with one peptide in the C-terminal part of the molecule. Antibody 8D4 reacted strongly with a number of α-gliadin peptides. The specificity of Abs 7C6 differed from those of the other Abs. These Abs recognized the lowest number of prolamin peptides and were targeted to only eight structural epitopes on ω-secalin and to only three epitopes on α-gliadin.

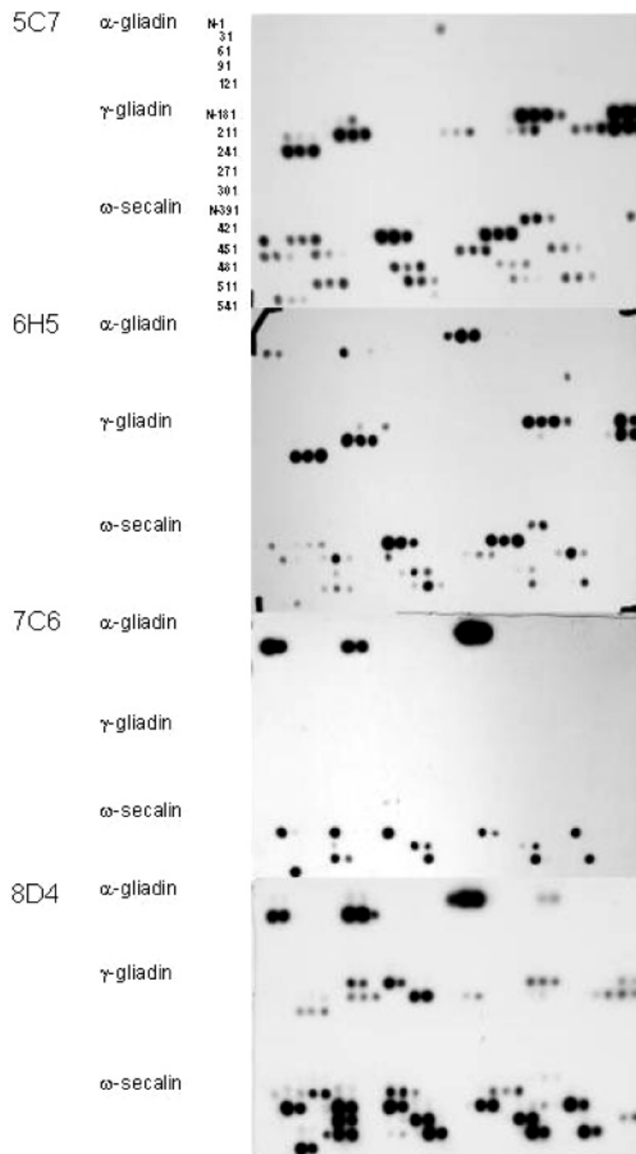

Figure 1. Illustrative photograph of individual reactivity of isolated IgG isotype of mouse monoclonal anti-gliadin antibodies (6H5, 5C7, 8D4, and 7C6) with peptides derived from α-gliadin (1–146), γ-gliadin (181–318), and ω-secalin (391–555).

The sequences recognized by all anti-gliadin Abs are summarized in **Table 2**. The peptides containing sequence motif QPFP, localized on α- and γ-gliadins as well as on ω-secalin, were recognized by all Abs, but with various binding intensities. In addition, the sequence motifs QQSFPQQ, located in several antigenic regions of ω-secalin and γ-gliadin molecules, and QQTFPQP, present in many peptides of γ-gliadin, were recognized by 6H5 and 5C7 Abs. In contrast, 7C6 Abs selectively recognized peptides containing two hexamer motifs, QPFP**X**Q (**X** = Q, L, P) and QPFRPQ peptides. The binding capacity of 7C6 Abs was completely lost by substitution of PQ by QP dipeptide in the QPFPPQ sequence.

The PEPSCAN technique used in the study is limited to the characterization of linear epitopes recognized by Abs. The use of overlapping dodecapetides enables the identification of antigenic epitopes composed of more than 10 amino acids. The relevance of conformational epitopes of dietary prolamins could be disputable, because exposure to a high temperature during food preparation, that is, baking or boiling, is known to affect molecular conformation. Moreover, prolamin-containing food

Table 2. Sequences of Gliadins (**A**, **B**) and Secalin (**C**) Recognized by Monoclonal Anti-gliadin Antibodies (6H5, 5C7, 8D4, 7C6)

no.	sequence	5C7	6H5	8D4	7C6	no.	sequence	5C7	6H5	8D4	7C6
(**A**) Peptides of α-Gliadin (No. 1–146)											
15	QFQGQQQPFP	2	2	3		32	QPQPFPPQLP		1	3	3
16	QGQQQPFPPQ		3	3	3	37	YPQPQPFRPQ		2	3	3
17	QQQPFPPQQP		3	3	3	38	QPQPFRPQQP			3	3
22	YPQPQPFPSQ			1		39	QPFRPQQPYP			3	
23	QPQPFPSQQP			1		114	SFQQPQQQYP		1		
31	FPQPQPFPPQ		1	3	3						
(**B**) Peptides of γ-Gliadin (No. 181–318)											
187	PQQQPFPQPQ		1	1		223	QLPFPQQPQQ			3	
188	QQPFPQPQQP	2		2		225	PQQPQQPFPQ	1			
189	PFPQPQQPFC		1			226	QPQQPFPQPQ	1		1	
190	PQPQQPFCQQ			3		227	QQPFPQPQQP	2		2	
191	PQQPFCQQPQ			2		230	PQQPQQPFPQ	1			
201	HHQPQQTFPQ	3	3	2		231	QPQQPFPQSQ	2			
202	QPQQTFPQPQ	3	3	1		232	QQPFPQSQQP	3		1	
203	QQTFPQPQQT	3	3	1		235	SQQPQQPFPQ	2			
204	TFPQPQQTYP	2	2			236	QPQQPFPQPQ	2		1	
208	YPHQPQQQFP	3	3	1		237	QQPFPQPQQQ	2		1	
209	HQPQQQFPQT	3	3	1		238	PFPQPQQQFP	3	3	2	
210	PQQQFPQTQQ	3	3	1		239	PQPQQQFPQP	3	3	1	
213	QTQQPQQPFP	2	2			240	PQQQFPQPQQ	3	3	1	
216	QPFPQPQQTF		3			242	FPQPQQPQQS		3		
217	FPQPQQTFPQ	3	3	1		243	QPQQPQQSFP	3	3	1	
218	QPQQTFPQQP	3	3	1		244	QQPQQSFPQQ	3	3	1	
219	QQTFPQQPQL	3		1		245	PQQSFPQQQQ	3		1	
222	QPQLPFPQQP			3							
(**C**) Peptides of ω-Secalin (No. 391–555)											
411	SPYQPQQPFP	3	2			472	QQPFSQPQQP			1	
412	YQPQQPFPQP	3	2			473	PFSQPQQPFP	2	1		
413	PQQPFPQPQQ	1				474	SQPQQPFPQQ	2	3	3	3
419	PIQPQQPFPQ	2				475	PQQPFPQQPG	1	1	3	
420	QPQQPFPQRP	2				486	LQPQQPFSQQ			3	
421	QQPFPQRPQQ	3		1		487	PQQPFSQQPQ			3	
422	PFPQRPQQPF		1	1		491	PQRPQQPFPQ	3			
423	PQRPQQPFPQ	3		1		492	RPQQPFPQQP	2	1	3	2
424	RPQQPFPQPQ	3		2		493	QQPFPQQPQQ	3	1	3	2
425	QQPFPQPQQQ	3	1	2		500	QPQQPFPLQP			3	
426	PFPQPQQQLP			1		501	QQPFPLQPQQ	2	1	3	2
427	PQFQQQLPLQ			1		508	QPQRPFGQQP			1	
430	LPLQPQQSFP	3	3	2		509	QRPFGQQPEQ			1	
431	LQPQQSFPQP	3	3	2		515	SQRPQQPFPL	3	1	2	
432	PQQSFPQPQH	3	1	1		516	RPQQPFPLQP	3	1	3	3
438	PQQPQQSFPQ	3	3	1		517	QQPFPLQPQQ	3	1	3	2
439	QPQQSFPQQP	3	3	1		521	QQPFSQPQQP			1	
440	QQSFPQQPQR	3	3	2		522	PFSQPQQPFP	2	1		
451	IPQQTQQPFP	3				523	SQPQQPFPQQ	2	3	3	3
452	QQTQQPFPLQ	3		3	3	524	PQQPFPQQPG	1	1	3	
453	TQQPFPLQPQ	1	1	3	2	530	IPQQPQQPFP	2			
454	QPFPLQPQQP			1	1	531	QQPQQPFPLQ	1	1	3	3
455	FPLQPQQPFP	2				532	PQQPFPLQPQ			3	
456	LQPQQPFPQQ	1	3	3	3	534	FPLQPQQPFP	3			
457	PQQPFPQQPQ	1		3	2	535	LQPQQPFPQQ	3	3	3	2
459	FPQQPQRPFA			1		536	PQQPFPQQPE	1		3	
460	QQPQRPFAQQ		1	3	3	541	QIISQQPQQP	3			
461	PQRPFAQQPE			3		542	ISQQPQQPFP	1			
466	QIISQQPFPL	3				543	QQPQQPFPLQ	1	1	3	3
467	ISQQPFPLQP	3	1	3	2	544	PQQPFPLQPQ			3	
468	QQPFPLQPQQ	3	1	3	2						

[a] Reactivity of antibodies with peptides was evaluated as 1 (weak), 2 (medium), and 3 (strong). The lack of a number represents no reactivity.

is subjected to digestion by enzymes in the stomach and intestine and cleaved to peptides.

In our study, Abs against gliadin were prepared using mice kept for two generations on GFD, that is, mice that did not have previous contact with gluten; we therefore assumed a higher response to gliadin in these animals. Of importance is the fact that several gliadin peptides recognized by our Abs were also target sequences recognized by anti-gliadin Abs of CLD patients. For example, the α-gliadin peptide motif QQQPFP was found to be recognized by both IgA and IgG anti-gliadin Abs isolated from the sera of CLD patients (*22*). This motif was also characterized as a target for R5 monoclonal anti-gliadin Abs (*23*, *24*). For this reason, we considered the QQQPFP antigenic motif as one of the immunodominant epitopes of gliadin recognized by CLD patients, as well as by gliadin-immunized mice. Moreover, the Abs binding the peptides containing the pentapeptide motif QQPFP and homologous sequences that occur repetitively in prolamins of wheat, rye, and barley were used in previously developed ELISAs (*25*, *26*).

Interestingly, the γ-gliadin sequence QPQQSFPQQQ, recognized also by mouse monoclonal Abs (6H5 and 5C7) in our study, was shown to stimulate the T cell response to gliadin

Table 3. Comparison of Various ELISA Tests for Detection of Gliadin in Food[a]

	Immunotech		Ridascreen		Riedel-de Haën	
sample	prolamins (mg/kg)	assessment (N/P)	prolamins (mg/kg)	assessment (N/P)	prolamins (mg/kg)	assessment (N/P)
1	<CC	N	<CC	N	5.56	N
2	135	P	113	P	12	N
3	20.8	N	10.2	N	24.8	N
4	5.83	N	2.06	N	<CC	N
5	5.83	N	1.94	N	14.5	N
6	104.1	P	>CC	P	178	P
7	10	N	6.46	N	<CC	N
8	7.49	N	11.2	N	15.9	N
9	6.81	N	24.5	N	7.05	N
10	<CC	N	<CC	N	6	N
11	<CC	N	<CC	N	<CC	N
12	4.12	N	<CC	N	<CC	N
13	98.7	BV	23.8	N	19.6	N
14	358	P	>CC	P	101	P
15	<CC	N	<CC	N	<CC	N
16	<CC	N	<CC	N	<CC	N
17	46.4	N	10.1	N	15.3	N
18	3.66	N	<CC	N	8.78	N
19	118	P	11.6	N	162	P

[a] P, positive, >100 mg of prolamins/kg of sample; N, negative, <100 mg/kg; BV, boundary value, ~100 mg/kg; CC, calibration curve; <CC, optical density of sample was lower than the lowest point of CC; >CC, optical density of sample was higher than the highest point of CC.

determined by a proliferation assay. Similarly, the peptide YPQPQPFRPQ of α-gliadin, recognized by 6H5 and 8D4, corresponds to the part of the α-gliadin peptide, PQPFRPQQPYPQPQPQ, stimulating T cell proliferate response (*27*−*29*).

Characteristics of the Newly Developed ELISA for the Detection of Gliadin. Monoclonal Abs 8D4 and 7C6 efficiently recognized peptides of α-gliadin, containing a toxic moiety (*30*−*32*). Hence, these Abs were used for the construction of a newly developed ELISA kit (Gliadin ELISA kit, IM3717, Immunotech). We therefore wanted to verify the efficiency of the kit by comparing it with corresponding commercial ELISA tests, that is, Riedel-de Haën 45213 and Ridascreen Gluten. Employing these tests we analyzed a set of 19 foodstuffs. **Table 3** shows the values of gliadin in the food measured by these three different tests. The positivity of the samples determined by the two commercial kits corresponded well with the results obtained by the newly developed kit; moreover, no substantial differences in quantitative estimation were found in most cases. Interestingly, the sensitivity of the novel ELISA test seems to be higher than the sensitivity of commercial kits used for comparison, as documented in samples 2, 13, and 19 (**Table 3**).

The main advantage of our ELISA for the detection of gliadin in food is the use of a combination of mouse monoclonal Abs based on their specificity, analyzed by PEPSCAN. We expect that the mentioned approach will increase the sensitivity of the test, as indicated during our pilot comparison among commercially available kits. This ELISA kit has been commercially available for almost 2 years.

In conclusion, PEPSCAN represents an efficient technique for analyzing Abs specificity, which in our case served for optimal selection of anti-gliadin Abs useful for the development of a sensitive and specific ELISA assay detecting gluten in foodstuffs.

ABBREVIATIONS USED

Abs, antibodies; CLD, celiac disease; ELISA, enzyme-linked immunosorbent assay; GFD, gluten-free diet; IMDM, Iscove's Modified Dulbecco's Medium; IR, intensity of reactivity; PBS, phosphate-buffered saline; RT, room temperature; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

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