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Similarity of Fine Specificity of IgA Anti-gliadin Antibodies between Patients with Celiac Disease and Humanized α1KI Mice

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ABSTRACT: Gliadins, and primarily α-gliadins containing several sequences such as aa 31−49, aa 56−88 (33-mer), aa 57−68, and aa 69−82, are critical in the induction of immune response or toxic reaction leading to the development of celiac disease (CLD). The role of IgA anti-gliadin antibodies (IgA AGA) is unknown. To this end, we prepared several humanized monoclonal IgA AGA using transgenic α1KI mice. Employing Pepscan with overlapping decapeptides of α-gliadin we observed a robust similarity between the specificity of humanized mouse monoclonal IgA AGA and IgA AGA from patients with florid CLD. The common immunodominant region included several sequential epitopes localized in the N-terminal part of α-gliadin (QFQGQQQPFPPQQPYPQPQPFP, aa 29−50, and QPFPSQQPYLQL, aa 47−58). Notably, IgA AGA produced by clones 8D12, 15B9, 9D12, and 18E2 had significant reactivity against sequences localized in the 33-mer, LQLQPFPQPQ (aa 56−65) and PQLPYPQPQPFL (aa 69−80). Humanized mouse monoclonal IgA AGA that have a known specificity are suitable as standard in ELISAs to detect serum IgA AGA of CLD patients and for studying the AGA pathogenic role in CLD, especially for analyzing the translocation of complex of specific IgA antibodies and individual gliadin peptides through enterocyte barrier.

KEYWORDS: α1KI mouse, IgA antibodies, gliadin, celiac disease, Pepscan

INTRODUCTION

Celiac disease (CLD) is a frequent, lifelong, small intestinal enteropathy with an incidence of 1:250−400, which is induced in genetically susceptible individuals after ingestion of wheat gluten. The duodenal and jejunal mucosa of patients with florid CLD is infiltrated by leukocytes and structurally remodeled. Villous flattening and crypt hyperplasia develop in the mucosa of these patients and cause malabsorption syndrome, diarrhea, abdominal pain, and weight loss. These symptoms, however, predominate in pediatric patients (accompanied by growth retardation), whereas latent and silent forms of CLD occur more often in adult patients.[1−3]

Gluten is a mixture of various proteins from the wheat kernel's endosperm that are classified on the basis of their solubility in ethanol: ethanol-insoluble (glutenins) and ethanol-soluble (gliadins) constitute the etiological agents of CLD.[4] Gliadins are grouped into three major types, α, γ, and ω, with molecular masses of 28−55 kDa.[5,6] Although CLD relapse is triggered by all of these gliadin types, a significant role in induction of the disease is played by α-gliadins containing several sequences such as aa 31−49, aa 56−88 (33-mer), aa 57−68, and aa 69−82 involved in pathogenetic mechanisms of the small intestine via their immunogenicity or toxicity to epithelial cells.[7−9]

The majority of CLD patients express the HLA−DQ heterodimer DQA1*0501/DQB1*0201 on antigen-presenting cells. In CLD the number of gliadin-specific HLA−DQ2- or HLA−DR4-restricted T-lymphocytes expands, and high titers of antibodies against gliadin and various autologous antigens are generated.[3] T-cell recognition could be increased after gliadin peptides have been deamidated by the enzyme tissue transglutaminase (tTG); wide variations in the degree of deamidation among peptides and among individual glutamine residues within each of them have been observed.[10]

Conversely, native gliadin peptides activate T-cells and innate immune cells in CLD patients.[11,12] Moreover, gliadin peptides effect direct damage in several biological models.[13−19]

Despite the growing knowledge of the immune system role in CLD pathogenesis, the therapy of the disease is based on the withdrawal of gluten and its related proteins from the diet of patients (gluten-free diet, GFD). After 6−12 months on a GFD, most CLD patients experience a restoration of intestinal mucosa and a reduction in the number of gliadin- and autoantigen-restricted lymphocytes and in the concentration of anti-gliadin antibodies (AGA) as well as of antibodies against autologous antigens, thus leading to clinical improvement.[1,2,9,20]

Unfortunately, a contamination of foodstuffs normally free of gluten by gliadin curtails one's adherence to a GFD. Long-lasting dietary gluten intake by CLD patients can lead to the development of complications, of which the most severe is refractory CLD and/or T-cell lymphoma.[21]

The diagnosis of CLD is based on the histological analysis of a duodenal/jejunal biopsy, which reveals the characteristic damage and remodeling of the mucosa in patients with suspected CLD

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Table 1. Amino Acid Sequence of α-Gliadin Described by Okita et al.[30]

1	VRVPVPQLQLQNPSQQQPQEQVPLVQEQQFQGQQQPFPPQQ
42	PYPQPQPFPSQQPYLQLQPFPQPQPFPPQLPYPQPQPFLPQQ
84	PYPQPQPQYSQPQQPISQQQQQQQQQQQQQQQQQQQILQQI
125	LQQQLIPCRDV VLQQHNIAHGSSQVLQEST YQLVQQLCCQQL
167	WQIPEQSRCQAIHNVVHAIILHQQHHHHQEQKQQLQQQQQQQ
209	QQLQQQQQQQQQQPLSQVSFQQPQQQYPSGQGFFQPSQQN
249	PQAQGSFQPQQLPQFEEIRNLALQTLPAMCNVYIPPYCSTTIAP
293	FGIFGTN

who have ingested gluten-containing foodstuffs. The extent of mucosal damage is evaluated per Marsh classification (I−IV): intra-epithelial lymphocytosis (I), proliferation of the crypts of Lieberkühn (II), partial or complete villous atrophy (III), and hypoplasia of the small bowel architecture (IV). The presence of increased serum concentrations of IgA AGA and IgA antibodies against tTG and endomysium is a strong indication for intestinal biopsy. The IgA AGA and anti-tTG antibodies tests serve to screen and monitor compliance to GFD.[22−24]

Florid CLD is characterized by elevated levels of IgA AGA; these antibodies, however, also exist in the sera of certain patients with other diseases, such as multiple myeloma, non-Hodgkin lymphoma, schizophrenia, autoimmune hepatitis, or primary biliary cirrhosis.[25−28] In contrast, the increasing prevalence of celiac children who are seronegative for AGA has been described.[29]

The detection of AGA by ELISA and its validity, however, depend on the antigens that are used and the internal standard (derived primarily from the sera of patients with florid CLD), and the sensitivity and specificity of tests can differ.

The genetic background of knock-in, humanized α1KI mice that bear a DNA segment that encodes the invariant part of the human immunoglobulin α-chain permits a precise analysis of antibody response to the food antigen gliadin and makes it possible to develop an understanding of the immune profile of these unique model animals.

We aimed to prepare several mouse monoclonal humanized IgA AGA using the transgenic mice and compared their fine specificity to the sequence of α-gliadin identified by Okita et al.[30] with that of serum IgA antibodies from patients with florid CLD. Despite the existence of a wide variety of α-gliadins differing in amino acid sequence that might be important for antibody generation,[31]we used this sequence to be able to compare our results with previously published experiments performed by the same technique.[32,33] The specificity of antibodies was analyzed by Pepscan using a set of synthetic overlapping decapeptides that corresponded to the entire sequence of α-gliadin.

MATERIALS AND METHODS

Patients. Serum of patients who had been newly diagnosed for CLD on normal gluten-containing diet (n = 12, mean age 21 years, range 18−27 years, 8 women and 4 men, all positive for IgA AGA, IgA anti-tTG, and anti-endomysial antibodies, and histology of duodenal mucosa according to Marsh III) was used. Sera of healthy blood donors (n = 12, mean age 23 years, range 19−41, 5 women and 7 men) were used as controls. Control sera were negative for the markers of CLD (IgA and IgG antibodies to tTG and AGA). The study was approved by the local Ethics Committee.

Preparation of Mouse Monoclonal Anti-gliadin Antibodies. α1KI mice[34] maintained on a standard diet were immunized intraperitoneally with two doses of 50−100 μg of crude gliadin (Sigma, Germany) in Freund's complete adjuvant (CFA) and a boosting dose without CFA. The levels of serum IgA AGA were tested repeatedly by direct two-step ELISA evaluated by optical density (OD) as described.[33,35] The OD of preimmune mouse sera was approximately 100, and in hyperimmune mouse sera it ranged from 1100 to 1400.

Cell fusion, selection of antibody-producing hybridomas, and their subsequent culture were performed as described.[36] Briefly, the spleen was dissected from the mouse and passed through a nylon filter generating a suspension of splenocytes. After several washes with Dulbecco's modified Eagle medium (DMEM) that contained 25 mM Hepes and L-glutamine (Cambrex, France), the cells were mixed with a suspension of SP20 (Ag14) myeloma cells at a 5:1 ratio. This mixture of both cell types was centrifuged, and the cell pellet was gently resuspended in 1 mL of polyethylene glycol (Roche, Switzerland) for 45 s and gently agitated.

Next, the medium was added dropwise to the cells, and the suspension was spun down. After the supernatant was aspirated, the cells were resuspended in hybridoma medium, that is, DMEM that contained fetal calf serum (10% v/v, Cambrex), hypoxanthine−thymidine (1% v/v, Sigma, Germany), and nonessential amino acids (5% v/v, Invitrogen, USA), and transferred (100 μL/well) into 96-well Nunclon-treated culture plates (Nunc, Denmark).

The day after fusion, hybridoma medium supplemented with HAT medium that contained aminopterin to select hybrid cells (1% v/v, Sigma, Germany) was added to each well (100 μL/well). After 15−20 days, supernatants from wells that contained clones were repeatedly tested for the presence of IgA AGA by ELISA. The studies were approved by the Animal Care and Use Committee of Limoges University and CNRS.

Pepscan Analysis. Tissue culture supernatants and mouse and human sera were diluted 1:100 or 1:50 to correspond to approximately 50% of OD on the titration curve of the IgA AGA.

The fine specificity of IgA AGA was analyzed using 146 decapeptides, corresponding to the entire amino acid sequence of α-gliadin (Table 1) described by Okita et al.,[30] accession number P04722 (http://www.uniprot.org). Individual peptides, overlapping by eight residues, were bound covalently via their C-termini to a cellulose membrane (Abimed, Langenfeld, Germany), spanning the entire sequence from the N- to the C-terminus. The peptides were prepared by automated spot synthesis on a cellulose membrane as previously described in detail.[37,38]

Pepscan analysis was performed as described.[33] Briefly, after being washed with methanol and TBS-T buffer (137.0 mM NaCl; 2.7 mM KCl; 50.4 mM Tris, 0.1% Tween, pH 8), the membranes were blocked in TBS-T that contained 2% low-fat milk, washed in TBS-T, and incubated with mouse serum, hybridoma supernatants, or human serum diluted in 2% low-fat milk in TBS-T overnight at 4 °C. The next day, the membranes were repeatedly washed in TBS-T and incubated for 1 h with sheep anti-human IgA antibodies (1:500). After another wash step, Pepscan membrane was developed using ECL reagents (Amersham, U.K.), and light emission was detected at various time points on X-ray film (Kodak, France).

The binding intensity of IgA antibodies to individual peptides of α-gliadin was assessed semiquantitatively on the basis of spot density and diameter. The data were digitized, and the image analysis was performed using a luminescence detector (Las 1000, Fujifilm). The binding

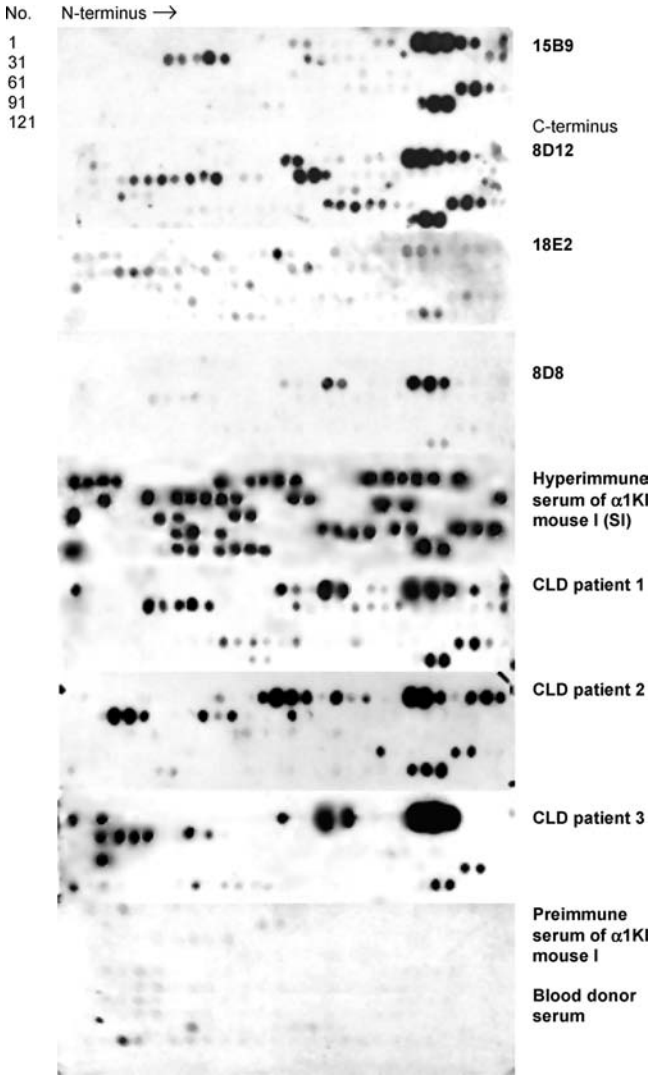

Figure 1. Illustrative picture of serum IgA antibody reactivity of several hybridoma clones (15B9, 8D12, 18E2, 8D8), hyperimmune serum of α1KI mouse I (SI), patients with florid celiac disease (CLD patients 1−3), premimmune serum of α1KI mice, and blood donor serum using Pepscan membrane containing the cohort of covalently bound, synthetic decapeptides completely covering the entire sequence of α-gliadin, each overlapping by eight amino acid residues. No. (α-gliadin peptide number).

intensity of monoclonal antibodies and antibodies from mouse sera was expressed as the intensity of reactivity (IR): strong (3), middle (2), and weak (1). The reactivity of IgA AGA of CLD patients was scored qualitatively as positive or negative and cumulatively expressed. Peptides bound by IgA antibodies of ≥ 50% of patients' sera were considered to be frequently recognized. For control purposes, the antibody specificity in each supernatant and serum was analyzed at various dilutions by Pepscan. Each sample and dilution was analyzed in at least three independent experiments.

RESULTS

We analyzed and compared the fine specificity of various humanized mouse monoclonal IgA AGA with the hyperimmune serum from donor mice and IgA AGA from patients with florid CLD by Pepscan using a set of overlapping synthetic peptides that encompassed the entire sequence of α-gliadin. We identified several antigenic epitopes on α-gliadin that were recognized by both serum IgA antibodies from hyperimmune donor α1KI mice and IgA antibodies from individual hybridoma clones that were derived from these mice.

Figure 1 shows the reactivity of monoclonal IgA antibodies produced by several hybridoma clones and IgA antibodies from hyperimmune serum of α1KI mice I (S I) and IgA antibodies of several patients with florid CLD with overlapping decapeptides covering the complete sequence of α-gliadin; preimmune serum of α1KI mice and blood donor serum were used as controls. The reactivity profiles of humanized IgA AGA of hyperimmune serum of two α1KI mice (I and II) and mouse monoclonal IgA AGA derived from these mice with overlapping decapeptides covering the complete sequence of α-gliadin are shown in Figures 2 and 3.

Quantitative and qualitative differences were observed between the specificity of IgA antibodies of hyperimmune mice and individual monoclonal antibodies tested, as shown in Table 2, in which are presented only peptides recognized by antibodies in at least one supernatant or serum. IgA antibodies from hyperimmune serum of donor α1KI mice reacted more robustly and with more epitopes on α-gliadin than an equivalent titer of hybridoma-produced IgA antibodies. Yet the number of α-gliadin peptides that were recognized by clones 8D12, 9D12, 18E2, and 15B9 was slightly lower compared with hyperimmune serum, and clones 8D8, 15A9, and, 20C2 reacted with substantially fewer peptides.

Although we also noted differences in the specificity and binding intensity of IgA antibodies among individual clones, for the majority of antibodies the most antigenic sites lay in the N- and C-terminal portions of α-gliadin. Only IgA antibodies from 8D8 and 15A9 bound well the peptide sequence VLQESTYQLVQQ (aa 149−160), in the central region of α-gliadin, which was recognized weakly by antibodies from clones of 8D12 and 8E12.

The cumulative map of the reactivity of serum IgA AGA from patients suffering from florid CLD with overlapping decapeptides covering the complete sequence of α-gliadin (Figure 4) shows two prominent peaks close to the N- and C-termini of α-gliadin. Frequently recognized epitopes in the N-terminal portion of α-gliadin contained several overlapping peptides often possessing antigenic core QPFP. Interestingly, another peptide, FPPQQPYPQP (aa 37−46), containing a related motif QPYP, was the most frequently recognized by IgA antibodies of CLD patients and also by IgA antibodies of clones 8D12 and 9D12.

The second immunodominant region of α-gliadin, recognized by IgA antibodies from >50% of CLD patients, lay in the C-terminal portion, formed by the sequence FQPQQLPQFEEIRNLALQ (aa 255−272, peptide 128−132). Moreover, the C-terminus of α-gliadin contained several peptides that were recognized with low frequency by serum IgA AGA from CLD patients, suggesting a rather individually specific character of patients' IgA antibodies reactivity against this part. Serum IgA antibodies from the majority of healthy donors tested did not react with peptides of α-gliadin or reacted weakly with some of the C-terminal peptides.

DISCUSSION

We prepared several humanized monoclonal IgA AGA using the humanized knock-in α1KI mice that harbored the DNA segment that encoded the invariant part of α-chain of human immunoglobulin and characterized and compared their fine specificity against α-gliadin with that of the serum IgA antibodies from patients with florid CLD by Pepscan. Our findings

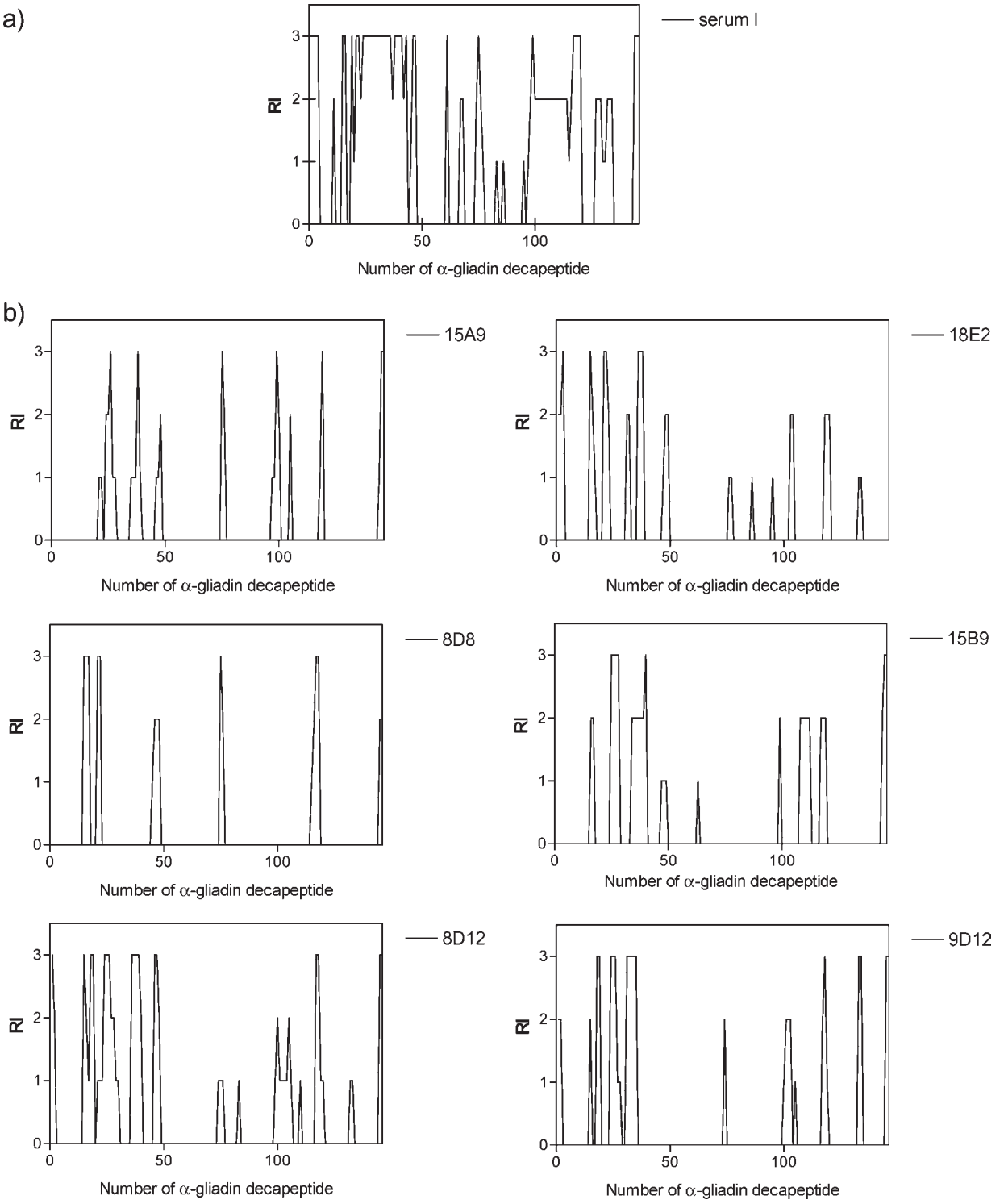

Figure 2. Reactivity profile of humanized IgA anti-gliadin antibodies of hyperimmune α1KI mouse I serum (a) and mouse monoclonal anti-gliadin antibodies produced by hybridoma clones 15A9, 8D8, 8D12, 18E2, 15B9, and 9D12 derived from this mouse with synthetic, overlapping, decapeptides completely covering the entire sequence of α-gliadin (1−146) using Pepscan technique (b).

regarding the antigenicity of the N- and C-terminal portions of α-gliadin are consistent with those obtained by Osman et al.[32]

Yet, we did not detect a peak of reactivity of patients' IgA antibodies against sequences in the center of α-gliadin.

Peptides that harbored the antigenic cores QPQPFP and QQQPFP were recognized by both IgA from CLD patients and humanized mouse monoclonal IgA antibodies, whereas peptides that contained QLQPFP or QGFFQP were recognized predominantly by IgA antibodies from a few clones. The only exception to this rule was peptide YPSGQGFFQP (aa 235−244), which was recognized by IgA antibodies of all clones tested. Peptides that contained the antigenic core PQQLPQ were

Table 2. Sequences of α-Gliadin Peptides Recognized by IgA Antibodies of Celiac Patients and Humanized IgA Anti-gliadin Antibodies of Hyperimmune Sera (S I, S II) and Hybridoma Clones (8D12, 8D8, 9D12, 18E2, 15A9, 15B9, 20C2)

no.	sequence	patients (%)	S I	8D12	8D8	9D12	18E2	15A9	15B9	S II	20C2
1	VRVPVPQLQL	25	3	3		2	2			2	2
2	VPVPQLQLQN	25	3	2		2	2			2	2
3	VPQLQLQNPS	25	3				3				
4	QLQLQNPSQQ	25	3								
10	QEQVPLVQEQ	25								2	
11	QVPLVQEQQF	42	2							2	
12	PLVQEQQFQG	50									
15	QFQGQQQPFP	58.3	3	3	3	2	3			3	1
16	QGQQQPFPPQ	16.7	3	2	3		2		2	2	
17	QQQPFPPQQP	58.3	1	1	3		1		2	1	
18	QPFPPQQPYP	75	1	3		3				2	
19	FPPQQPYPQP	92	3	3		3					
20	PQQPYPQPQP	33.3	1							2	
21	QPYPQPQPFP	58.3	3	1	3		3	1		3	
22	YPQPQPFPSQ	33.3	3	1	3		3	1		3	1
23	QPQPFPSQQP	42	2	1			2			3	2
24	QPFPSQQPYL	50	3	3		3		2		3	3
25	FPSQQPYLQL	50	3	3		3		2	3	3	3
26	SQQPYLQLQP	33.3	3	3		3		3	3	3	3
27	QPYLQLQPFP	16.7	3	2		1		1	3	2	2
28	YLQLQPFPQP	33.3	3	2		1		1	3		
29	QLQPFPQPQP	42	3	1						2	
30	QPFPQPQPFP	42	3	1						2	
31	FPQPQPFPPQ	8.3	3			3	2			2	1
32	QPQPFPPQLP	8.3	3			3	2			1	1
33	QPFPPQLPYP	0	3			3				1	
34	FPPQLPYPQP	0	3			3			2	2	2
35	PQLPYPQPQP	0	3			3		1	2	2	2
36	LPYPQPQPFL	8.3	3	3			3	1	2	2	1
37	YPQPQPFLPQ	8.3	2	3			3	1	2	1	1
38	QPQPFLPQQP	8.3	3	3			3	3	2	3	
39	QPFLPQQPYP	33.3	3	3				1	2	2	2
40	FLPQQPYPQP	42	3	2					3	3	
41	PQQPYPQPQP	33.3	3								
42	QPYPQPQPQY	8.3	2								
43	YPQPQPQYSQ	0	3								
45	QPQYSQPQQP	42	1		1						
46	QYSQPQQPIS	42	3	3	2			1			
47	SQPQQPISQQ	42	3	3	2		1	1	1		
48	PQQPISQQQQ	25	1	2	2		2	2	1		
49	QPISQQQQQQ	8.3									
56	QQQQQQQQQI	8.3									
57	QQQQQQQILQ	8.3									
58	QQQQQILQQI	8.3									
61	LQQILQQQLI	0	3								
63	LQQQLIPCRD	0							1		
67	RDVVLQQHNI	25	2								
68	VVLQQHNIAH	0	2								
74	SQVLQESTYQ	0	2	1		2					
75	VLQESTYQLV	8.3	3	1	3			3			
76	QESTYQLVQQ	8.3	2	1	2		1	2			
77	STYQLVQQLC	0	1				1				
82	CQQLWQIPEQ	25									

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Table 2. Continued

no.	sequence	patients (%)	S I	8D12	8D8	9D12	18E2	15A9	15B9	S II	20C2
83	QLWQIPEQSR	25	1	1							
84	WQIPEQSRCQ	25									
86	EQSRCQAIHN	0	1				1				
95	QQHHHHQEQK	8.3	1				1				
97	HHQEQKQQLQ	0	1					1			
98	QEQKQQLQQQ	0	2					1		2	
99	QKQQLQQQQQ	8.3	3	1				3	2	2	
100	QQLQQQQQQQ	0	2	2		1		2		1	
101	LQQQQQQQQQ	8.3	2	1		2				1	
102	QQQQQQQQLQ	8.3	2	1		2					
103	QQQQQQLQQQ	8.3	2	1		2	2			2	
104	QQQQLQQQQQ	0	2	1			2			2	
105	QQLQQQQQQQ	0	2	2		1		2		1	
106	LQQQQQQQQQ	8.3	2	1							
107	QQQQQQQQQP	0	2								
108	QQQQQQQPLS	16.7	2						2	1	
109	QQQQQPLSQV	25	2						2	2	
110	QQQPLSQVSF	16.7	2	1					2		
111	QPLSQVSFQQ	8.3	2						2		
112	LSQVSFQQPQ	8.3	2						2		
113	QVSFQQPQQQ	33.3	2								
114	SFQQPQQQYP	41.7	2								
115	QQPQQQYPSG	41.7	1		1						
116	PQQQYPSGQG	33.3	2		2						
117	QQYPSGQGFF	16.7	3	3	3	2			2	1	1
118	YPSGQGFFQP	16.7	3	3	3	3	2	1	2	2	2
119	SGQGFFQPSQ	16.7	3	1		1	2	3	2	1	2
120	QGFFQPSQQN	16.7	3	1			2				3
127	GSFQPQQLPQ	33.3	2								
128	FQPQQLPQFE	50	2								
129	PQQLPQFEEI	50	2								
130	QLPQFEEIRN	58.3	1								
131	PQFEEIRNLA	58.3	1							2	
132	FEEIRNLALQ	66.6	2	1							
133	EIRNLALQTL	0	2	1		3	1				
134	RNLALQTLPA	8.3	2			3	1				
144	STTIAPFGIF	33.3	3					1	2		
145	TIAPFGIFGT	33.3	3	3	2	3		3	3		
146	IAPFGIFGTN	25	3	3	2	3		3	3		

recognized primarily by patient sera, but WQIPEQ-containing peptides were not bound frequently by patients' IgA antibodies or IgA antibodies from any clone. Notably, the peptide IAPFGIFGTN (aa 290−299), to which IgA antibodies of α1KI mouse I hyperimmune serum and IgA antibodies from all clones reacted strongly, had been identified in our laboratory by Krupičková et al.[39] as a sequence that inhibits the binding of AGA, isolated from CLD patients, to calreticulin and enterocytes.[40]

We noted similarities between the fine specificity of humanized monoclonal IgA AGA and IgA AGA from patients with florid CLD for the N-terminal portion of α-gliadin. Immunodominant epitopes that are characteristic of CLD patients forming part of the sequence QFQGQQQPFPPQQPYPQPQPFPSQQPYLQL (aa 29−58) encompassed epitopes that were recognized by IgA antibodies from hyperimmune donor α1KI mice and by monoclonal IgA antibodies from clones 8D12 and 9D12. The sequence of immunodominant epitopes in CLD patients (QFQGQQQPFPPQQP, aa 29−42) overlapped partially with epitopes that were recognized by IgA antibodies from clones 8D8, 18E2, and 15B9.

Conversely, the cumulative reactivity of IgA antibodies from clones 8D8, 9D12, 18E2, and 15B9 covered the dominant epitopes in the N-terminus of α-gliadin, bound by serum IgA antibodies from patients with florid CLD. In contrast to the relatively high homology among antigenic epitopes that were recognized by IgA antibodies from patients with florid CLD and humanized mouse monoclonal IgA AGA against N-terminus of α-gliadin, there was little similarity among the epitopes that were recognized in its C-terminus. The overlapping peptides that constituted the sequence FQPQQLPQFEEIRNLALQ (aa 255−272), recognized by IgA antibodies from >50% of CLD patients, were bound weakly by IgA antibodies of hyperimmune sera from α1KI mice but not by antibodies from any clone.

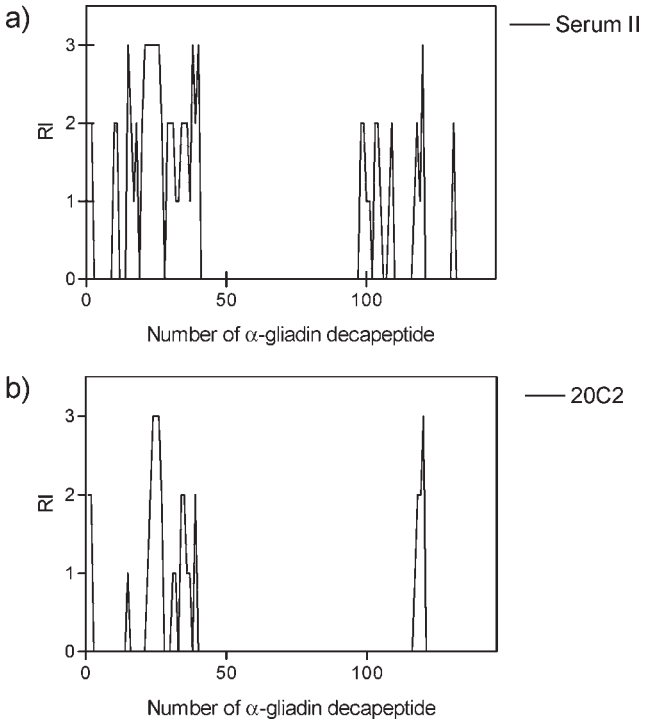

Figure 3. Reactivity profile of humanized IgA anti-gliadin antibodies of hyperimmune α1KI mouse II serum (a), and mouse monoclonal anti-gliadin antibodies produced by hybridoma clone 20C2 derived from this mouse with synthetic, overlapping, decapeptides completely covering the entire sequence of α-gliadin (1−146) using Pepscan technique (b).

In contrast, the peptides that corresponded to the LQLQPFPQPQ (aa 56−65) and PQLPYPQPQPFL (aa 69−80) segments of the 33-mer of α-gliadin were recognized frequently by the majority of humanized IgA antibodies from clones and hyperimmune sera. The specificity of the former for the 33-mer was verified by ELISA at several dilutions of supernatant (data not shown).

In our study, we detected at least some IgA antibody response against polyglutamine regions in C-terminal half, whereas polyglutamine regions in N-terminal half were only rarely recognized. Despite the high similarity among the sequences of polyglutamine peptides in both halves, they differed in the content of other amino acids in various positions. This fact is probably the reason for the differences in reactivity of tested antibodies.

We also documented a more robust antibody response in α1KI mice against α-gliadin compared with monoclonal IgG1 AGA from Balb/C mice that were maintained on GFD for two generations and subcutaneously immunized by crude gliadin in a previous study.[33] Humanized α1KI mouse serum IgA AGA and monoclonal IgA AGA that were derived from B-cells from these animals reacted with a substantially broader spectrum of antigenic epitopes on α-gliadin than the mouse monoclonal IgG1 antibodies. We speculate that the reason for this phenomenon is the unique immunologic phenotype of transgenic α1KI mice. Intraperitoneal immunization with gliadin also likely contributed to the induction of a vigorous antibody response and determinant spreading.

Despite the fact that CLD is considered to be a T-cell-mediated disease, IgA antibodies to gliadin and autoantibodies against tTG and endomysium are its serological hallmark. The reason for the generation of IgA AGA is not fully understood and could be associated with genetic background, increased permeability of mucosal barrier, and/or failure of oral tolerance. However, it is known that IgA AGA

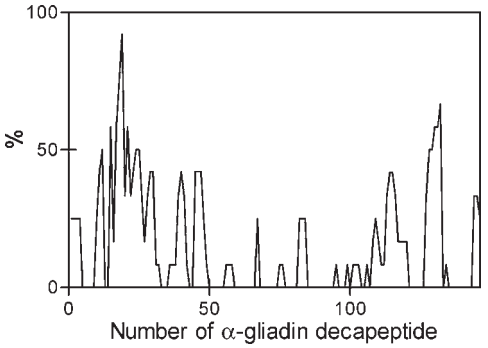

Figure 4. Profile of cumulative data of serum IgA antibody reactivity of patients with florid celiac disease with synthetic, overlapping, decapeptides completely covering the entire sequence of α-gliadin (1−146). The data are expressed as percent of sera in tested group (n = 12) recognizing the individual peptides using the Pepscan technique.

detected in serum can be partially generated by B-cells in gut mucosa as a response to gliadin uptake.[41,42] We suppose that IgA AGA could play a role in the process of antigenic (gliadin) exclusion.

In conclusion, our results suggest that α1KI mice are a suitable tool for producing highly specific humanized IgA antibodies. This is underscored by the resemblance of the specificity of humanized monoclonal IgA antibodies to α-gliadin that are derived from these mice with IgA from patients with florid CLD. A suitable combination of humanized mouse IgA AGA with known specificity can supplement animal (polyclonal/monoclonal) or human serum IgA antibodies as standard in ELISAs to detect serum IgA AGA of CLD patients and can serve for testing the presence of gliadin in foodstuffs in GFD. Monoclonal antibody production methods provide a stable source of antibodies. Hence, the chief advantages of a test that is based on humanized monoclonal antibodies that have known specificities are its simplicity, reproducibility, sensitivity, and specificity.

Furthermore, humanized mouse IgA AGA are suitable for use in detailed studies of their role in the pathogenesis of CLD and its associated diseases. Antibodies that are directed against the 33-mer and against other immunodominant peptides of α-gliadin can be used to analyze the translocation of the complex of gliadin with specific antibodies through enterocytes similarly as published by Heyman and Menard[43] and their processing by antigen-presenting cells in CLD patients.

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ABBREVIATIONS USED

CLD, celiac disease; AGA, anti-gliadin antibodies; IgA, immunoglobulin A; HLA, human leukocyte antigen system; tTG, tissue

transglutaminase; GFD, gluten-free diet; CFA, complete Freund's adjuvant; TBS-T, tris(hydroxymethyl)aminomethane buffered saline with Tween 20; HAT, hypoxanthine, aminopterin, thymidine; OD, optical density; IR, intensity of reactivity; ECL, enhanced chemiluminiscence.

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