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Original article

Identification of IgE-binding epitopes on gliadins for patients with food allergy to wheat

Background: Food allergy to wheat induces different symptoms as atopic eczema/dermatitis syndrome (AEDS), urticaria and more severe reactions as wheat-dependent exercise-induced anaphylaxis (WDEIA). Different gliadin classes are involved in this allergy but IgE-binding epitopes are known only on ω_5-gliadins and for WDEIA cases.
Objectives: The aim of the study was to identify IgE-binding epitopes on several gliadin classes and for several patients with different symptoms and ages.
Methods: Eleven sera were analysed by pepscan with overlapping synthetic peptides.
Results: Sera from five patients with anaphylaxis, urticaria or WDEIA, displayed strong IgE-binding to sequential epitopes of the repetitive domains of αβ, γ, ω_2 or ω_5-gliadins with two immunodominant epitopes on ω_5-gliadin and a consensus motif of the type QQX_1PX_2QQ (X_1 being L, F, S or I and X_2 Q, E or G). One patient allergic to deamidated wheat proteins also had IgE to a repetitive peptide of γ and ω_2-gliadins of the type QPQQPFP. Sera from four patients with AEDS detected no linear epitopes on gliadins, despite the fact that they contained specific IgE to α, β, γ or ω-gliadins. One child with AEDS recognized cysteine-containing sequences in the nonrepetitive domains of αβ and γ-gliadins.
Conclusion: B epitopes in wheat allergy were different from B epitopes of coeliac disease. Differences exist in IgE-binding epitopes between patients with food allergy to wheat. IgE from those suffering from WDEIA, anaphylaxis and urticaria detected sequential epitopes in the repetitive domain of gliadins whereas IgE from AEDS patients probably recognized conformational epitopes.

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Key words: AEDS; gliadins; pepscan analysis; sequential B-epitopes; WDEIA; wheat allergy.

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Wheat is a major food of our diet, however it can be responsible of food-allergic reactions in children and adults. The main symptom of food allergy to wheat in children is atopic eczema/dermatitis syndrome (AEDS) and it occurs in a context of multiple food allergies (1–3). In adults, wheat can elicit severe reactions and the most frequent and increasing symptom is wheat-dependent exercise-induced anaphylaxis (WDEIA) (2, 4–7).

Major proteins of wheat flour, gliadins and glutenins, as well as proteins of the albumin/globulin fraction are involved in food allergy to wheat (8–12). Gliadins appeared as important allergens and different classes seemed to be involved in the IgE response. Different classes of gliadins αβ-, γ- and ω- have been defined according to protein sequences and electrophoretic mobility. Their sequence includes a repetitive and a non-repetitive domain. Gliadins present important sequence homologies particularly in their repetitive domain rich in glutamine and proline (13). Omega-gliadins lack cystein and repetitive motives cover almost the entire sequence. Several distinct ω-gliadins were identified. Kasarda et al. (14) described ω_1, ω_2 and ω_5-gliadin components according to their electrophoretic mobility in acid polyacrylamide gel electrophoresis and N-terminal sequences beginning with KEL, ARE/Q and SRL, respectively. Omega5 or fast-moving ω-gliadin components differ from other ω-gliadin components by a higher glutamine content. The special role of ω_5-gliadins in WDEIA had been demonstrated by several studies (5, 15, 16). We observed IgE binding to αβ-, γ- and ω-gliadins for wheat allergic children and adults (12).

Only two studies have been carried out as yet to determine IgE-binding epitopes in food allergy to wheat.

Abbreviations: AS, anaphylactic shock; AEDS, atopic eczema/dermatitis syndrome; Asth, asthma; B, bread; G, gluten; U, urticaria; WDEIA, wheat-dependent exercise-induced anaphylaxis; WF, wheat flour.

One was restricted to WDEIA cases and to the ω_5-gliadin allergen (7) and the other identified for a small number of patients with AEDS an IgE binding motif on low molecular weight glutenin subunits (17). Knowledge of more B-cell epitopes would help to understand the molecular basis of allergic reactions to wheat. Here, we report the first attempt to identify IgE-binding epitopes on several gliadin types and for several groups of patients differing by their symptoms and ages. We also included in the study a patient allergic to chemically modified wheat proteins. We used overlapping synthetic peptides (18) covering entire or partial sequences of different gliadin types.

Methods

Population studied

Ten patients with wheat allergy were selected on the basis of a clinical diagnosis (Table 1). Clinical symptoms were: AEDS (19) isolated or associated to asthma (five cases), exercise-induced anaphylaxis (three cases), anaphylactic shock (one case) and urticaria (one case) (Table 1). A supplementary case was a patient allergic to deamidated wheat proteins who tolerated natural wheat flour (patient 30) (20). Sensitization to wheat was established by positive prick-in-prick tests to wheat and or positive Cap System Rast.

Skin prick tests

The negative and positive controls were physiological saline and 9% codeine phosphate, respectively. Positive results were defined as a wheal whose diameter was 75% that of the codeine control, the physiological saline control being negative. Prick-in-prick tests were carried out with natural foods deposited onto the skin, pricking through the powder (21). The materials were a wheat flour bought in the market (WF), a flour-mill gluten (G) and a wheat extract (Allerbio Laboratory, Varennes en Argonne, France).

Table 1. Characteristics of patients whose sera were used for pepscan analysis

Patients	Age (years)	Symptom	PIP (mm)/ natural wheat	DBPCFC: reactive dose (mg protein equivalent)	Gliadin fractions detected by IgE in RAST or ELISA
21	2	AEDS + Asth	2.5	4000	α, β, γ, ω
27	5	AEDS + Asth	11	290	α, β, γ, ω
50	2	AEDS	10	1000	α, β, γ, $\omega_{1,2}$, ω_5
53	3	AEDS	4	ND (LT+)***	γ, $\omega_{1,2}$, ω_5
56	2	AEDS	2	1560	α, β, γ, $\omega_{1,2}$, ω_5
3	65	U	6	9000*	α, β, γ, ω
30**	24	U	4	8600	γ, $\omega_{1,2}$
41	3	AS	13	ND	α, β, γ, $\omega_{1,2}$, ω_5
31	37	WDEIA	11.5	ND	ω_5
58	44	WDEIA	2	1500	ω_5
61	41	WDEIA	6	8600	α, β, $\omega_{1,2}$, ω_5

PIP, prick-in-prick; DBPCFC, double-blind placebo-controlled food challenge; AS, anaphylactic shock; AEDS, atopic eczema/dermatitis syndrome; Asth, asthma; WDEIA, wheat-dependent exercise-induced anaphylaxis; U, urticaria; ND, not done.
*Positive effect of avoidance diet.
**Patient allergic to deamidated wheat proteins.
***LT, labial test.

Challenge

Allergy was diagnosed by placebo-controlled double-blind food challenges (22). Oral food challenge was carried out after 3 weeks of a strict WF avoidance diet. Tests were carried out during a 24-h hospitalization period during which blood pressure and pulse were monitored, lung auscultation performed, peak expiratory flow rate measured and any modifications in skin and mucosa recorded. The positive criteria included the following objective clinical signs: drop in blood pressure, tachycardia, urticaria or angioedema, rash, cough, sibilant rales on auscultation, drop in PEF and/or FEV_1 > 20%, rhinorrhea, conjunctival redness, diarrhoea and vomiting, exacerbation of eczema with modification of SCORAD in the event of atopic dermatitis (23). The only subjective criterion that was taken into account was abdominal pain in children, as it has been observed earlier that increasing the dose in such cases constantly elicits objective signs and sometimes serious ones (data not shown). Oral food challenge was first performed single-blind in children and double-blind in adults. The first cases underwent a standard gradual five-dose increase starting at 15 mg WF to reach a total dose of 7110 mg WF (24). Later, one or two doses were administered; about 30 g of wheat flour, 1–5 g of gluten, 1–50 g of bread (except one patient who received 150 g). The vehicle used was stewed apple or mashed potatoes. The placebo was either cooked corn or rice flour. If results were negative, a second, open food challenge was carried out with crust and breaddough (B) added to flour, in order to be palatable and to take into account possible changes in allergenicity induced by cooking, that could be related to the case of the patient (25). Two adults underwent tests that included physical activity: running for 5 min to 1 h after eating the triple combination WF–G–B.

The study was approved by the committee for protection of human subjects in biomedical research of Cochin hospital (Paris). Patients or their parents gave their informed consent to the clinical procedure of diagnosis.

IgE-binding to solid-phase synthetic peptides (Pepscan)

Eleven sera from wheat allergic patients and four sera from non-atopic subjects used as controls were subjected to the pepscan analysis. A preliminary screening of specific IgE to the different components of gliadins was performed by RAST and immunoblotting (12) or ELISA (Battais F, Courcoux P, Popineau Y, Kanny G, Moneret–Vautrin DA, Denery-Papini S, unpublished data) (Table 1).

Decapeptides, overlapping on eight amino acids, spanned the sequence (excluding signal peptide) of αβ-gliadin [accession number P04725 (26)] of 299 amino acids, of γ-gliadin [accession number P08453 (27)] of 308 amino acids and of ω_2-gliadin [accession number Q9FUW7 (28)] of 261 amino acids and fragments of ω_5-gliadin (29) (five fragments of 25, 19, 25, 26 and 31 amino acids) (Fig. 1). Decapeptides were prepared by automated spot synthesis according to the procedure described by Frank (30) and were covalently fixed by their C-terminal end on a cellulose membrane (Abimed, Lanenfeld, Germany). After washing 5 min with methanol and three times 5 min with a Tris 50 mM buffer pH 8.0 containing 0.05% Tween 20 (v/v) (TBS/T), membranes were blocked with 2.5% (w/v) skimmed dried milk and 5% (w/v) sucrose in TBS/T during 1 h, washed in TBS-T, and incubated overnight with sera from patients diluted 1/20 (or 1/40 for serum 41) in blocking buffer. Each step was performed at room temperature under agitation. After further washing, anti-human IgE antibodies (ε chain) labelled with peroxidase (P0295; Dako, Glostrup, Denmark) were added in blocking buffer for 1 h at 1/1000 or 1/10 000. In a first procedure, membranes were incubated in a chemiluminescent substrate

αβ-Gliadin :

1 VRVPVPQLQPQNP⌈SQQQP**QEQVPLVQQQQFPGQQQQ**FPPQQPYPQPQPFPSQQPYL
57 QLQPFPQPQPFPPQLPYPQPQSFPPQQPYPQQQPQYLQPQQPISQQQAQQQQQQQQ
113 QQQQQQC⌋ILQQILQQQLIPCRDVVLQQHNIHASSQVLQQSTYQLLQQLCCQQLLQIPEQ
172 SQCQAIHNVAHAIIMHQQQQQQEQKQQLQQQQQQQQLQQQQQQQQQQPSS**QVSF**
227 **QQPQQQ**YPSSQVSFQPSQLNPQAQGSVQPQQLPQFAEIRN***LALQTLPAMC***NVYIPPH
284 CSTTIAPFGISGTN

γ-Gliadin :

1 NIQVDPSGQVQW⌈**LQQQLVPQLQ**QPLSQQPQQTFPQPQQTFPHQPQQVPQPQ**QPQQP**
57 **FLQPQQPFPQQPQQPFP**QTQ**QPQQPFP**Q**QPQQPFP**QTQ**QPQQPFP**Q**QPQQPFP**QTQ
113 **QPQQPFP**QLQ**QPQQPFP**QP**QQQLPQPQQPQQSFPQQQRP**FIQPS⌋LQQQLNPCKNILLQ
170 QSKPASLVSSLWSIIWPQSDCQVMRQQCCQQ***LAQIPQQLQC***AAIHSVVHSIIMQQQQQQ
229 QQQQGIDIFLPLSQHEQVGQGSLVQGQGIIQPQQPAQLEAIRSLVLQTLPSMCNVYVPPE
289 CSIMRAPFASIVAGIGGQ

ω2-Gliadin:

1 ARELNPSNKEL⌈QSPQQSFSYQQQPFPQQPYPQQPYPSQQPYPSQQP**FPTPQQQFPE**
57 **QSQQ**PFTQPQQPTPI**QPQQPFP**QQPQ**QPQQPFPQPQQPF**PW**QPQQPFP**Q**TQQSFPL**
113 **Q**PQQPFPQ**QPQQPFP**QPQLPFPQQSEQIIPQQLQQPFPL**QPQQPFP**Q**QPQQPFP**QP**Q**
170 **QPIPVQPQQ**SFPQQSQQSQQPFAQPQQLFPELQQPIPQ**QPQQPFP**L**QPQQPFP**Q**QP**
226 **QQPFP**QQPQQSFPQQPQQPYPQQQPY⌋GSSLTSIGGQ

ω5-Gliadin fragments:

Fragment 1: SRLLSPRGKELHT⌈PQEFQFPQQQ
Fragment 2: IS**QQPQQLPQQQIPQQP**
Fragment 3: **FHQQQLPQQQFPQQQFPQQQFPQ**
Fragment 4: LHQPEQFPQQQQFPQPQQFPQLPI
Fragment 5: LTQQQFPR**PQQSPEQQQFPQQQFPQQ**PPQ

Figure 1. Gliadin sequences used in pepscan analysis and position of some peptides bound by IgE from patients with food allergy to wheat. Peptides detected by patients suffering from urticaria, anaphylaxis or WDEIA: characters in bold and underlined. Peptides detected by patient 27 suffering from AD: characters in bold and broken underlined. Peptides detected by patient 30 allergic to deamidated wheat proteins: characters in bold. Limits of repetitive domains: ⌈⌋.

(SuperSignal West Pico kit; Pierce, Rockford, IL, USA) for 5 min without agitation and then luminescence was measured by an optical camera in real time. In a second procedure, chemiluminescent substrate (SuperSignal West Dura Extended Duration Substrate; Pierce) was added, luminescence detected on a Kodak X-OMAT AR-5 film (Pharmacia, Uppsala, Sweden) and measured by densitometry. According to Osman, the positivity was semi-quantitatively assessed (18). The strongest binding of specific IgE to a peptide was the reference (31). Sera were classified as strongly positive (≥60%) or positive (20–60%). Control sera were totally negative, however, a weak binding under 20% was not interpreted.

Results

No IgE binding could be detected for the control sera. Examples of pepscan membrane are presented in Fig. 2.

Peptides bound by IgE of patients suffering from AEDS

No peptide was bound by IgE antibodies from four children suffering from AEDS (patients 21, 50, 53, 56). A fifth child suffering from AEDS (patient 27) exhibited IgE binding to five peptides in the C-terminal non-repetitive domain of αβ-gliadin sequence and to one peptide in the same domain of γ-gliadin (Table 2, Fig. 1). All these peptides except one contained a cystein. This serum detected no epitopes on the two *ω*-gliadin sequences.

Peptides bound by IgE from patients suffering from urticaria or anaphylaxis

Sera of adult patients with WDEIA or urticaria (patients 3, 31, 61, 58) and of one child with anaphylaxis (patient 41) displayed IgE-binding to peptides belonging solely, except one, to the repetitive domains of the gliadins analysed (Table 2, Figs 1 and 2).

These five patients displayed IgE-binding to a large number of peptides present on several fragments of ω_5-gliadin. Antibodies from all these patients reacted with peptides FHQQQLPQQQ or QQQLPQQQFP on fragment 3 and IgE from four of them reacted with the same peptide, QQQFPQQQFP, on fragments 3 and 5. Several other peptides were detected by IgE from two or

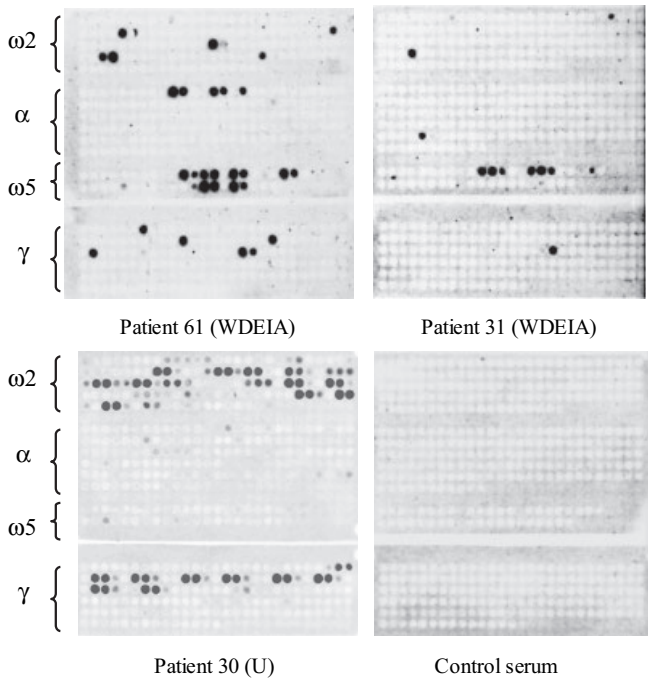

Figure 2. Examples of IgE binding to synthetic peptides covering sequences of αβ, γ and ω_2-gliadins and fragments of ω_5-gliadin on pepscan membranes for two patients with food allergy to wheat (31 and 61), one patient allergic to a wheat isolate (30) and one control serum.

three patients on fragments 2 and 5 of ω_5-gliadin. Homologies could be observed between these various epitopes and sequence alignment revealed a consensus motive of type QQX_1PX_2QQ, with X_1 being either L, F, S or I and X_2 being either Q, E or G (Table 3). Only one serum (patient 41) reacted with peptides of fragment 4 and no epitopes were found on fragment 1 corresponding to the N-terminal end of ω_5-gliadin.

Four of these five sera also reacted to a set of peptides localized in a region close to the N-terminal end of αβ-gliadin sequence (Table 2, Fig. 1). Two overlapping epitopes on this protein could be aligned with the consensus sequence found on ω_5-gliadin (Table 3). The last serum (from patient 31) detected a single peptide in the non-repetitive C-terminal end of the sequence.

On the γ-gliadin sequence, IgE from two patients reacted with peptides localized near the N-terminal end and IgE from three patients with peptides localized further in the γ-gliadin repetitive domain (Table 2, Fig. 1). Comparison of these peptides showed a consensus sequence, of the type QQX_3X_4PQ, different from that found on ω_5 and αβ-gliadins (Table 3).

Nine different IgE-binding regions were detected on ω_2-gliadin repetitive sequence (Table 2). Some peptides were detected by antibodies from two or three patients (Fig. 1) and a consensus motive of type $QQPX_5PX_6Q$ could be found for epitopes recognized by patients 3, 41, 61 and 58 (Table 3).

Peptides bound by IgE from patient 30 with food allergy to deamidated wheat proteins

Patient 30 displayed strong IgE binding to numerous peptides on ω_2 and γ-gliadin sequences (Figs 1 and 2). Comparison of all these peptides showed an unique epitope present in the repetitive region of γ- and ω_2-gliadins: QPQQPFP. This epitope is repeated about 10 times in the repetitive regions from these two gliadins. It is not present on the sequences analysed for αβ and ω_5-gliadins where only very weak binding were observed.

Discussion

Wheat B-epitopes are not uniformly distributed all over the peptide sequence. Pepscan analysis revealed strong IgE-binding of all five patients with WDEIA, anaphylaxis, or urticaria to sequential epitopes localized in the repetitive domains of αβ, γ, ω_2-gliadins and particularly to some repeats on ω_5-gliadin fragments (Fig. 1). Two fragments of ω_5-gliadin were detected and contained two immunodominant epitopes: QQQLPQQQ, QQQFPQQQ being detected, respectively, by five and four patients. Fewer peptides were detected on αβ, γ and ω_2-gliadins. Comparison of epitopes on ω_5 and α-gliadins pointed out a consensus sequence of the type QX_1PX_2QQ whereas different motives were detected on γ and ω_2-gliadins (QQX_3X_4PQ and $QQPX_5PX_6Q$, respectively). These results are in good agreement with those of Matsuo et al. (7) who identified four dominant epitopes QQIPQQQ, QQFPQQQ, QQSPEQQ and QQSPQQQ on ω_5-gliadin for patients with WDEIA, with critical amino acids at position 1 and 4 to 7. It is thus interesting to see that patients from Japanese and French origin recognized the same epitopes. Our study was not restricted to WDEIA patients and suggested that other patients with wheat allergy suffering from urticaria or anaphylaxis not related to exercise also had IgE antibodies directed to these epitopes. Our study also demonstrates that although most immunodominant epitopes were found on ω_5-gliadin, IgE from these patients can also bind peptides on other gliadins. Patient 30 suffering from urticaria after consumption of deamidated wheat proteins also presented strong IgE binding to sequential epitopes of the repetitive domain of γ and ω_2-gliadins but did not react with fragments from ω_5-gliadins. His antibodies recognized an unique epitope: QPQQPFP in the repetitive domain of these gliadins. This epitope is different from those recognized by patient 3 suffering from urticaria due to non-modified wheat proteins and from peptides detected by other sera. This peptide is repeated about 10 times in the sequence of the native gliadins.

Most patients with AEDS did not react with any peptides. Patient 27 reacted with cysteine-containing peptides localized in the non-repetitive domain of αβ

Table 2. Sequences of peptides bound on αβ, γ and ω_2-gliadins and ω_5-gliadin fragments in pepscan analysis by IgE from patients with food allergy to wheat

Patients	Age (year)/symptoms	α/β-gliadin	γ-gliadin	ω_2-gliadin	ω_5-gliadin
27	5/AEDS + asth	QILQQQLIPC	LAQIPQQLQC		
		LQIPEQSQCQ			
		QEQKQQLQQQ			
		LALQTLPAMC*			
		YIPPHCSTTI			
41	3/AS	**VQQQQFPGQQ**	QQQLPQPQQP	FPTPQQQFPE	QQQLPQQQFP
		QQQFPGQQQQ		TPQQQFPEQS	QLPQQQFPQQ
		QFPGQQQQFP		**QQQFPEQSQQ**	QQFPQQQFPQ
				IQPQQPFPQQ	FPQQQFPQQQ
				QTQQSFPLQ	QQQFPQQQFP
				QQSFPLQPQQ	QQFPQQQFPQ
				LQPQQPFPQQ	EQFPQQQQFP
				QQSFPQQSQQ	QQQQFPQPQQ
				FAQPQQLFPE	QSPEQQQFPQ
				QPQQLFPELQ	PEQQQFPQQQ
				QQLFPELQQP	QQQFPQQQFP
				QQPQQPFPQQ	**QFPQQQFPQQ**
				QQPQQSFPQQ	QQQFPQQPPQ
3	65/U	VQQQQFPGQQ		SPQQSFSYQQ	**FHQQQLPQQQ**
		QQQFPGQQQQ		QQSFSYQQQP	**FPQQQFPQQQ**
					QQQFPQQQFP
					QQQFPQQQFP
58	44/WDEIA	**QEQVPLVQQQ**	**LQQQLVPQLQ**	QQPIPVQPQQ	FHQQQLPQQQ
			QQLVPQLQQP		PQQSPEQQQF
31	37/WDEIA	QVSFQQPQQQ	**QQSFPQQQRP**	FPTPQQQFPE	**QQPQQLPQQQ**
				QQPIPVQPQQ	**PQQLPQQQIP**
					QLPQQQIPQQ
					FHQQQLPQQQ
					QQQLPQQQFP
					QLPQQQFPQQ
					QQQFPQQQFP
					QQQFPQQQFP
61	41/WDEIA	**QEQVPLVQQQ**	LQQQLVPQLQ	TQPQQPTPIQ	**QQPQQLPQQQ**
		QVPLVQQQQF	QTQQPQQPFP	QTQQSFPLQP	PQQLPQQQIP
		QQQFPGQQQQ	QTQQPQQPFP	**QQSEQIIPQQ**	QLPQQQIPQQ
		QFPGQQQQFP	QTQQPQQPFP	QPQQPIPVQP	**LPQQQIPQQP**
		QQQQFPPQQP	QPQQSFPQQQ	**QQPIPVQPQQ**	**FHQQQLPQQQ**
			QQSFPQQQRP	PELQQPIPQQ	QQQLPQQQFP
					QQQFPQQQFP
					QQFPQQQFPQ
					PRPQQSPEQQ
					PQQSPEQQQF
					PEQQQFPQQQ
					QQQFPQQQFP

*Bold characters represent strongly positive peptides.

and γ-gliadins. Sera from these patients reacted in RAST or ELISA with some native purified gliadins (Table 1) and some of them also in immunoblotting after disulfide bond reduction (not shown). The lack of response in pepscan analysis suggested that in cases of AEDS the main IgE-binding epitopes on gliadins are conformational. The epitopes detected by IgE from patient 27 were different from that (QQQPP) identified by Tanabe et al. (17) on low molecular weight glutenin subunits for four wheat allergic AEDS patients.

The comparison of B epitopes in wheat allergy and in coeliac disease deserves consideration. In wheat allergy, B-cell epitopes have been detected in the repetitive domain of gliadins as it was described also for coeliac disease (18). However, if the domain is the same, the sequences are not similar. Sequences recognized by IgA antibodies from coeliac patients on α-gliadin are: QXQPFP, WQIPEQ, QGXFQP or PQQLPQ and on γ-gliadins: QPQQPF (18). However, patient 30 allergic to deamidated gluten reacted with an epitope (QPQQPFP) on ω_2 and γ-gliadins similar to that recognized by IgA of coeliac patients on γ-gliadins (no peptides of ω-gliadins were tested with coeliac sera) (18). In coeliac disease, the deamidation of toxic peptides by the tissue transglutaminase increases the antigenicity of gliadin peptides resistant to gastrointestinal digestion

Table 3. Sequence alignment for IgE-binding epitopes on the different gliadins and consensus motives (in bold characters)

ω_5- and α-gliadins	γ-gliadin	ω_2-gliadin
FHQ**QQLPQQQ**		
Q**QQLPQQQ**FP		
FPQ**QQFPQQQ**		
Q**QQFPQQQ**FP		
QQFPQQQFPQ		
P**QQSPEQQ**F		
PRP**QQSPEQQ**		
QQP**QQLPQQQ**		
P**QQLPQQQ**IP		**QQPIPVQ**PQQ
LPQ**QQIPQQP**	**QQQLPQ**PQQP	PEL**QQPIPQQ**
PEQ**QQFPQQQ**	LQ**QQLVPQ**LQ	TQP**QQPTPIQ**
	QQLVPQLQQP	QQP**QQPFPQQ**
VQQ**QQFPGQQ**	QP**QQSFPQ**QQ	IQP**QQPFPQQ**
Q**QQFPGQQ**QQ	**QQSFPQ**QQRP	LQP**QQPFPQQ**
QQX$_1$PX$_2$QQ	**QQX$_3$X$_4$PQ**	**QQPX$_5$PX$_6$Q**

(32) and increases IgA binding to these peptides (18). Serum of patient 30 showed a weak IgE reactivity in ELISA against native ω_2 and γ-gliadins and a strong reactivity against deamidated gluten. Thus its IgE response would probably be more intense against a deamidated form of this QPQQPFP sequence and peptides with substitutions of glutamine by glutamic acid residues need to be tested.

The present results also show a difference in IgE reactivity between patients with different symptoms, to be confirmed on a larger number of patients. Precisely, linear B epitopes were involved in WDEIA whereas for wheat allergic patients with AEDS, B epitopes on $\alpha\beta$, γ and ω-gliadins seemed conformational. In gliadins, different reactivity towards the two structural domains may be related to asymmetry in their conformation. Indeed, computer modelling indicates that the non-repetitive domain of $\alpha\beta$ and γ-gliadins has a more compact structure due to the presence of three and four disulfide bonds, respectively (33, 34) and is likely to contain conformational epitopes whereas the repetitive domains of gliadins possess a more extended and flexible structure. Their loose loops may allow recognition of linear epitopes by specific IgE.

May this difference in epitope profile be linked with a difference in allergy outcome? Indeed, WDEIA seems persistent whereas most of wheat allergic children with AEDS recovered within a few years (D.A. Moneret-Vautrin, personal communication). This hypothesis is drawn on the basis of data on milk and egg allergies where correlations were suggested between IgE responses to linear epitopes and allergy persistence and between IgE responses to conformational epitopes and recovery (35–37).

In conclusion, we have identified several IgE-binding linear epitopes on $\alpha\beta$,γ and ω-gliadins for wheat allergic patients. A remarkable point is that epitopes in wheat allergy are different from epitopes reactive with IgA from coeliac patients, documenting the clinical knowledge that there are no overlaps between these two pathologies, so that an association would be an exceptional random case. Such an association has not been published up to now. In wheat allergy, differences have been demonstrated between patients suffering from AEDS and those suffering from WDEIA, anaphylaxis and urticaria. Patients with WDEIA, urticaria and anaphylaxis, mainly adults, showed strong IgE-binding to sequential epitopes of the repetitive domains. The absence of IgE to linear epitopes in children with AEDS leads to hypothesize specific responses to conformational epitopes. Further data on allergy evolution might help to find relationships between some epitopes and allergy persistence as in the case of egg and milk allergies.

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