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Identification of the IgE-binding Epitope in ω-5 Gliadin, a Major Allergen in Wheat-dependent Exercise-induced Anaphylaxis*

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Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a severe IgE-mediated allergic reaction provoked by the combination of wheat-ingestion with intensive physical exercise over the next few hours. Among wheat proteins, ω-5 gliadin, which is one of the components of fast ω-gliadin, has been reported as a major allergen in the anaphylaxis. In this study, we detected IgE-binding epitopes within the primary sequence of ω-5 gliadin using arrays of overlapping peptides synthesized on derivatized cellulose membranes. Sera from four patients with WDEIA having specific IgE to the fast ω-gliadin were used to probe the membrane. Seven epitopes, QQIPQQQ, QQLPQQQ, QQFPQQQ, QQSPEQQ, QQSPQQQ, QQYPQQQ, and PYPP, were detected within the primary sequence of ω-5 gliadin. By using sera of 15 patients, 4 of them, QQIPQQQ, QQFPQQQ, QQSPEQQ, and QQSPQQQ, were found to be dominant epitopes. Mutational analysis of the QQIPQQQ and QQFPQQQ indicated that amino acids at positions Gln^1, Pro^4, Gln^5, Gln^6, and Gln^7 were critical for IgE binding. These results will provide a useful tool for developing safer wheat products in addition to diagnostic and immunotherapy techniques for WDEIA.

Food-dependent exercise-induced anaphylaxis is a distinct form of food allergy induced by physical exercise (1). Food items such as shrimp (2), hazelnut (3), buckwheat (4), corn (5), and celery (6) are responsible for the development of food-dependent exercise-induced anaphylaxis. However, of all of the various kinds of food, wheat is reported to be the allergen with the highest frequency in Japan (7). Symptoms are typically generalized urticaria and severe allergic reactions such as shock or hypotension. Because of this serious reaction, it is important to determine the causative food to avoid the allergic reaction. A challenge test consisting of ingestion of the assumed food followed by intense physical exercise is the only reliable method to determine the causative food and to diagnose the disease. However, the challenge test is not always safe because in some cases the test induces an anaphylactic shock. In addition, the most reliable treatment for this disease is to avoid taking the causative food or, alternatively, to take a rest after meals. However, in the case of wheat allergy, elimination causes a decline in the quality of life for the patients. Thus, an *in vitro* diagnostic method as well as hypoallergenic wheat is necessary for patients with wheat-dependent exercise-induced anaphylaxis (WDEIA).[1]

Recent studies have revealed the IgE-binding epitopes of several food allergens including egg (8), milk (9, 10), soybean (11), and peanut (12), whereas the IgE-binding epitopes for wheat allergen are controversial. Wheat protein is composed of water/salt-soluble proteins and water/salt-insoluble proteins. Proteins in the water/salt-soluble fraction, such as α-amylase inhibitor, peroxidase, glycerinaldehyde-3-phosphate dehydrogenase, serpin, and triosephosphate isomerase, have been considered to be major allergens in patients with bakers' asthma (13–15). On the other hand, Sandiford *et al.* (16) showed that α-gliadin and fast ω-gliadin (water/salt-insoluble wheat proteins) are the allergens associated with bakers' asthma. Watanabe and co-workers (17, 18) found that low molecular weight (LMW) glutenin, one of the water/salt-insoluble proteins, is the major allergen for patients allergic to wheat, and identified QQQPP motif as an IgE-binding epitope. Maruyama *et al.* (19) reported that α-gliadin and γ-gliadin in addition to LMW glutenin are the allergens for patients with wheat allergies (19). These observations indicate that a variety of wheat proteins are allergenic.

In terms of WDEIA, in our previous studies (20–22), we identified wheat fast ω-gliadin as a major allergen and found that the specific IgE against fast ω-gliadin cross-reacts to γ-gliadin and slow ω-gliadin (20–22). Fast ω-gliadin is also called 1B-type ω-gliadin (23) or ω-5-type gliadin (24, 25) and can be divided into several peaks by reversed-phase high performance liquid chromatography (24). Palosuo *et al.* (26, 27) identified ω-5 gliadin, a component of fast ω-gliadin, as a major allergen in WDEIA. They revealed that the ω-5 gliadin is also an allergen in children with an immediate-type allergy to wheat (27) and that transglutaminase-mediated cross-linking of a pepsin-trypsin-digested ω-5 gliadin causes a marked increase in IgE-binding both *in vitro* and *in vivo* (28). In this study, we analyzed the linear IgE-binding epitopes of ω-5 gliadin by using synthetic peptides.

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[1] The abbreviations used are: WDEIA, wheat-dependent exercise-induced anaphylaxis; LMW, low molecular weight; HMW, high molecular weight; TBST, 50 mM Tris-buffered saline, 1% Tween 20, pH 7.4; Fmoc, *N*-(9-fluorenyl)methoxycarbonyl; RAST, radioallergosorbent test.

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TABLE I
Results of dot blot analysis

The PVDF membranes spotted with the purified wheat proteins were incubated with 10% sera of the patients. An enhanced chemiluminescence system was used for detection of the IgE antibodies reacting to the wheat proteins. The resulting lights were visualized on autoradiography film. After scanning the film, the intensities of spots intensities were calculated using the image-analyzing software. Ua, units of allergen and specific IgE; α, α-gliadin; β, β-gliadin; γ, γ-gliadin; Fω, fast ω-gliadin; Sω, slow ω-gliadin.

	Total IgE	Gluten RAST	Spot intensity							
			α	β	γ	Fω	Sω	HMW	LMW	Gluten
	IU/ml	*Ua/ml*								
WDEIA patients										
1	146	<0.35	1	1	0	133	2	6	8	47
2	228	1.32	9	9	5	360	53	125	111	238
3	21.9	0.51	0	0	1	21	6	9	9	22
4	1779	5.34	63	9	6	176	77	1586	154	1724
5	44.5	1.55	5	0	0	696	33	9	161	290
6	176	1.22	2	0	0	911	13	78	46	166
7	184	2.3	5	1	0	47	12	4	36	55
8	169	<0.35	0	0	0	181	8	34	14	28
9	136	1.33	10	4	2	308	23	6	11	119
10	397	3.83	4	3	8	233	226	6	81	147
11[a]	14,723	3.33	460	1170	646	981	76	1227	183	1331
12	434	14.0	6	11	13	455	24	7	19	158
13	150	<0.35	3	3	1	12	67	171	14	438
14	104	1.79	0	0	0	153	50	4	38	39
15	171	1.54	3	1	1	41	40	20	37	36
Non-WDEIA controls										
16	57	<0.35	2	0	0	6	13	0	2	34
17	110	<0.35	5	13	6	20	0	9	3	82
18	100	<0.35	1	8	3	22	4	1	0	33
19	500	<0.35	8	15	5	19	1	2	2	98
20	31	<0.35	0	0	0	2	10	1	5	3
21	1600	<0.35	17	10	16	39	72	32	71	42
22	7.2	<0.35	0	1	2	11	50	17	18	4
23	26	<0.35	2	3	5	11	24	7	29	72
24	9.8	<0.35	18	12	7	78	17	4	17	94
25	290	<0.35	4	17	7	23	22	8	51	33
26	41	<0.35	7	14	9	14	48	39	68	53
27	310	<0.35	0	0	0	7	24	17	11	6
28	310	<0.35	3	1	0	34	36	10	8	23
29	47	<0.35	0	0	0	8	37	8	8	3
30	21	<0.35	15	44	58	21	89	158	189	61
31[a]	16,100	1.64	10	2	0	33	81	6	177	113
32[a]	10,990	0.81	0	2	3	16	57	6	100	126
33[a]	656	<0.35	0	4	2	10	21	302	11	24
34[a]	1900	<0.35	9	7	6	46	123	62	92	104
35[a]	580	<0.35	1	3	0	10	47	13	25	41
36[a]	370	<0.35	2	3	3	5	29	1	16	38

[a] Patient with atopic dermatitis.

EXPERIMENTAL PROCEDURES

Subjects—Sera were collected from 15 patients with WDEIA who had recurrent episodes of anaphylaxis and a positive-provocation test to exercise after wheat ingestion, 15 healthy subjects without episodes of food allergy and 6 patients with atopic dermatitis without episodes of WDEIA. The sera were stored at −80 °C until use. Specific IgE antibodies for gluten in the sera were determined by the radioallergosorbent test system (CAP-RAST, Amersham Biosciences) (29).

Dot-blotting—Wheat α-gliadin, β-gliadin, γ-gliadin, fast ω-gliadin, slow ω-gliadin, high molecular weight (HMW) glutenin, and LMW glutenin were prepared as described previously (16, 30). Specific IgE antibodies in the sera for α-gliadin, β-gliadin, γ-gliadin, fast ω-gliadin, slow ω-gliadin, HMW glutenin, and LMW glutenin were detected using a method described previously with a slight modification (20). 2 μg of each gliadin, glutenin, and gluten (NAKARAI TESQUE, Kyoto, Japan) were spotted on polyvinylidene fluoride membrane (Immobilon, Millipore) and blocked with 5% skim milk in TBST. The membrane was washed three times with TBST for 10 min and then incubated with 10% of the patient's serum overnight at 4 °C. After washing with TBST, the membrane was incubated with goat anti-human IgE horseradish peroxidase-conjugate (BIOSOURCE) for 1 h at room temperature. The membrane was washed three times with TBST for 10 min, and the IgE antibodies reacting to the wheat proteins were visualized using ECL Plus Western blotting detection reagents (Amersham Biosciences). The resulting light was detected on autoradiography film (Hyperfilm ECL, Amersham Biosciences). After scanning the film, the spot intensities were measured using the Gel-Pro Analyzer software (Media Cybernetics, Inc).

Peptide Synthesis and IgE-binding Assay—Four DNA sequences of ω-5 gliadin fragments (GenBank™ accession numbers BE590673, BQ608902, BQ805830, and BQ245835) were selected from the wheat-expressed sequence tags data base. These sequences were aligned, and the assumed amino acid sequence of ω-5 gliadin was constructed (Fig. 1). Based on the assumed amino acid sequence of the ω-5 gliadin, the individual peptides were synthesized on a membrane using a SPOTs kit (Sigma), which contains a derivatized cellulose membrane (SPOTs membrane). Fmoc amino acids, by coupling Fmoc amino acids according to the manufacturer's instructions. After washing with methanol, the SPOTs membrane was blocked overnight at 4 °C with blocking buffer (Sigma). The membrane was washed with TBST for 10 min at room temperature and then incubated overnight at 4 °C with 10% patient's serum. The bound IgE antibodies were detected as described above with goat anti-human IgE horseradish peroxidase conjugate by using ECL Plus Western blotting detection reagents. The SPOTs membrane was used repeatedly after the following procedure. The membrane was washed in order with water, *N*,*N*-dimethylformamide (WAKO Chemical, Osaka, Japan), Regeneration buffer A (8 M urea, 10% sodium dodecyl sulfate), and Regeneration buffer B (10% (v/v) acetic acid, 50% (v/v) ethanol), rinsed with methanol twice for 10 min at room temperature, and dried.

RESULTS

Dot-blotting for Purified Gliadins and Glutenins—Specific IgE reacting with the purified gliadins and glutenins in the sera of 15 patients with WDEIA and 21 control subjects were determined by dot-blotting. Table I shows the serum IgE levels,

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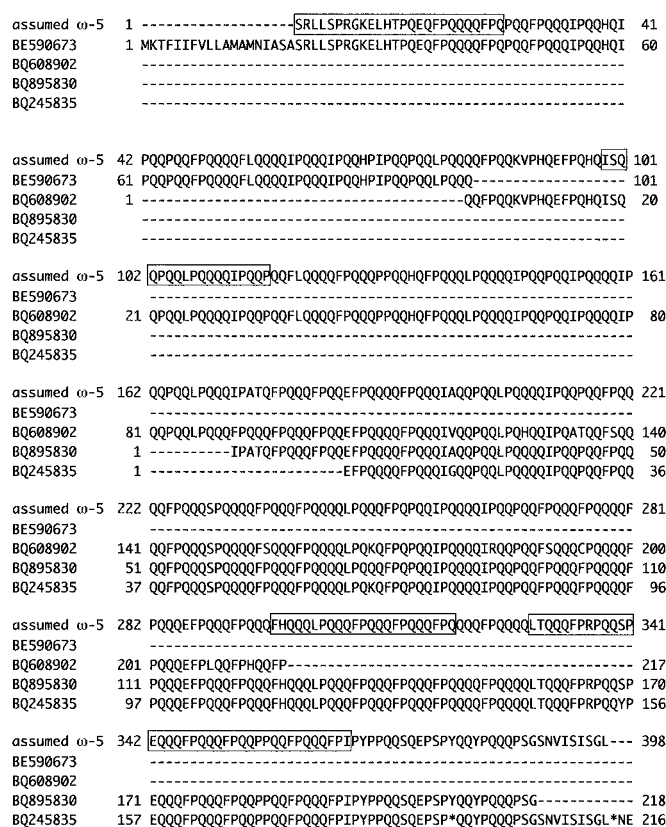
the CAP-RAST value for gluten, and the result of the dot-blotting analysis. In the data of CAP-RAST for gluten, sensitivity and specificity for diagnosis of WDEIA were 80 (12/15) and 90% (19/21), respectively, when the cutoff value for a positive result was set at 0.35 units of allergen-specific IgE per ml. In the data of dot-blotting for gluten, when a cutoff value was set at 100 based on the data of control subjects, sensitivity and specificity were 60 (9/15) and 85% (18/21), respectively, both of which were worse than those of CAP-RAST for gluten. Additionally, the values of spot intensity for gluten were also not always parallel to that of CAP-RAST. This may be attributed to the unstable technique of dot-blotting.

Among the individual data of spot intensity for purified wheat proteins, the strongest reaction was observed with the fast ω-gliadin in 12 of 15 patients with WDEIA (patients 1, 2, 3, 5, 6, 7, 8, 9, 10, 12, 14, and 15), confirming the data reported by Palosuo *et al.* (26, 27). The remaining three patients (patients 4, 11, and 13) had specific IgE reacting predominantly to HMW glutenin. In contrast, the strongest reaction was observed with slow ω-gliadin in 10 of 21 control subjects and LMW glutenin was the second (6 of 21 patients). In 4 of 21 control subjects, the strongest reaction was also observed with fast ω-gliadin as in the patients with WDEIA; however, the values of spot intensities were relatively low compared with those of patients with WDEIA.

Multiple IgE-binding Epitopes in the ω-5 Gliadin—Because no cDNA and genomic DNA sequence of ω-5-type gliadins has been reported, we collected four DNA fragment sequences of ω-5 type gliadins from the wheat-expressed sequence tags data base based on the *N*-terminal and internal protein sequences (23, 24). We then aligned these sequences and constructed an assumed amino acid sequence of ω-5 gliadin (Fig. 1). The molecular mass of the assumed ω-5 gliadin was calculated at 48-kDa, suggesting that it was almost full-length because the determination of molecular mass by matrix-assisted laser desorption ionization time-of-flight revealed a range of 44–55 kDa for the ω-5-type gliadins (24).

66 overlapping peptides were synthesized by the SPOTs method to determine which regions of the assumed ω-5 gliadin were recognized by the serum IgE of the patients with WDEIA. Each peptide was 13 amino acids long and offset by six amino acids. Individual sera from four patients (patients 1, 2, 3, and 5) whose serum had specific IgE to fast ω-gliadin predominantly were selected and used to map the IgE-binding region in the assumed amino acid sequence of ω-5 gliadin. Fig. 2 summarizes the IgE-binding peptides of each of the four patients. Many IgE-binding peptides were detected; however, most of these positive sequences were overlapped among the tested patients. From the results of the alignment of the IgE-bound peptide sequences, we expected that common epitope sequences are QQIPQQQ (peptides 10, 26, and 43), QQFPQQQ (peptides 5, 8, 21, 32, 36, 37, 39, 40, 45, 46, 47, 49, 51, 52, 53, 54, 58, and 60), and QQLPQQQ (peptides 13, 18, 23, 24, 28, 34, 41, and 50). Peptides 38, 57, and 64 also reacted to the sera commonly in four patients, respectively. The sera of patient 3 reacted with two other peptides (peptides 61 and 62), indicating proper epitopes to this patient.

Determination of the Epitope Sequence and Critical Amino Acids—For determination of the exact amino acid sequence of IgE-binding regions, synthetic peptides comprising 10 amino acids (offset by one amino acid) were further synthesized with respect to the IgE-bound peptides (peptides 26, 28, 38, 47, 57, 61, 62, and 64, respectively) derived from the results of Fig. 2. These peptides then were probed with the sera of randomly selected patients with WDEIA (patients 1, 2, 3, and 5). The immunoblotting result of peptide 26 with the serum of patient


```
assumed ω-5  1 ------------------SRLLSPRGKELHTPQEQFPQQQQFPQPQQFPQQQIPQQHQI  41
BE590673     1 MKTFIIFVLLAMAMNIASASRLLSPRGKELHTPQEQFPQQQQFPQPQQFPQQQIPQQHQI  60
BQ608902       ------------------------------------------------------------
BQ895830       ------------------------------------------------------------
BQ245835       ------------------------------------------------------------

assumed ω-5 42 PQQPQQFPQQQQFLQQQQIPQQQIPQQHPIPQQPQQLPQQQQFPQQKVPHQEFPQHQISQ 101
BE590673    61 PQQPQQFPQQQQFLQQQQIPQQQIPQQHPIPQQPQQLPQQQ------------------- 101
BQ608902     1 ----------------------------------------QQFPQQKVPHQEFPQHQISQ  20
BQ895830       ------------------------------------------------------------
BQ245835       ------------------------------------------------------------

assumed ω-5 102 QPQQLPQQQQIPQQPQQFLQQQQFPQQQPPQQHQFPQQQLPQQQQIPQQPQQIPQQQQIP 161
BE590673        ------------------------------------------------------------
BQ608902     21 QPQQLPQQQQIPQQPQQFLQQQQFPQQQPPQQHQFPQQQLPQQQQIPQQPQQIPQQQQIP  80
BQ895830        ------------------------------------------------------------
BQ245835        ------------------------------------------------------------

assumed ω-5 162 QQPQQLPQQQIPATQFPQQQFPQQEFPQQQQFPQQQIAQQPQQLPQQQQIPQQPQQFPQQ 221
BE590673        ------------------------------------------------------------
BQ608902     81 QQPQQLPQQQFPQQQFPQQQFPQQEFPQQQQFPQQQIVQQPQQLPQHQQIPQATQQFSQQ 140
BQ895830      1 ----------IPATQFPQQQFPQQEFPQQQQFPQQQIAQQPQQLPQQQQIPQQPQQFPQQ  50
BQ245835      1 -----------------------EFPQQQQFPQQQIGQQPQQLPQQQQIPQQPQQFPQQ  36

assumed ω-5 222 QQFPQQQSPQQQQFPQQQFPQQQQLPQQQFPQPQQIPQQQQIPQQPQQFPQQQFPQQQQF 281
BE590673        ------------------------------------------------------------
BQ608902    141 QQFPQQQSPQQQQFSQQQFPQQQQLPQKQFPQPQQIPQQQQIRQQPQQFSQQQCPQQQQF 200
BQ895830     51 QQFPQQQSPQQQQFPQQQFPQQQQLPQQQFPQPQQIPQQQQIPQQPQQFPQQQFPQQQQF 110
BQ245835     37 QQFPQQQSPQQQQFPQQQFPQQQQLPQKQFPQPQQIPQQQQIPQQPQQFPQQQFPQQQQF  96

assumed ω-5 282 PQQQEFPQQQFPQQQFHQQQLPQQQFPQQQFPQQQFPQQQFPQQQQLTQQQFPRPQQSP 341
BE590673        ------------------------------------------------------------
BQ608902    201 PQQQEFPLQQFPHQQFP------------------------------------------ 217
BQ895830    111 PQQQEFPQQQFPQQQFHQQQLPQQQFPQQQFPQQQFPQQQFPQQQQLTQQQFPRPQQSP 170
BQ245835     97 PQQQEFPQQQFPQQQFHQQQLPQQQFPQQQFPQQQFPQQQFPQQQQLTQQQFPRPQQYP 156

assumed ω-5 342 EQQQFPQQQFPQQPPQQFPQQQFPIPYPPQQSQEPSPYQQYPQQQPSGSNVISISGL--- 398
BE590673        ------------------------------------------------------------
BQ608902        ------------------------------------------------------------
BQ895830    171 EQQQFPQQQFPQQPPQQFPQQQFPIPYPPQQSQEPSPYQQYPQQQPSG------------ 218
BQ245835    157 EQQQFPQQQFPQQPPQQFPQQQFPIPYPPQQSQEPSP*QQYPQQQPSGSNVISISGL*NE 216
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FIG. 1. **Alignment of putative amino acid sequence of ω-5-type gliadin selected from wheat expressed sequence tags DNA data base (GenBank™ accession numbers BE590637, BQ608902, BQ895830, and BQ245835).** Amino acid sequences including ω-5 gliadin reported by DuPont *et al.* (23) are *boxed*. *Asterisks* indicate the stop codon based on the DNA sequence.

1 was shown in Fig. 3. The *boxed area* indicates the core epitope sequence. The IgE-binding epitopes on ω-5 gliadin obtained from all four patients identified in the same manner were summarized in Table II. All of the sera reacted to QQIPQQQ, QQLPQQQ, QQFPQQQ, QQSPEQQ, QQSPQQQ, and QQYPQQQ with the exception of patient 5 whose serum failed to react to QQLPQQQ. Additionally, the serum of patient 3 had specific IgE to PYPP.

The critical amino acids for IgE-binding in two epitopes, QQIPQQQ and QQFPQQQ, were determined by synthesizing epitope peptides with single amino acid mutation at each position followed by probing with individual sera from four patients (patient 1, 2, 3, and 5). The data of the immunoblot strip in patient 1 were representatively shown in Fig. 4. No binding of the serum IgE was observed when the alanine was substituted at the amino acid positions Gln^1, Pro^4, Gln^5, Gln^6, and Gln^7, respectively, indicating that these amino acids are critical for IgE binding in both epitopes. The remaining sera of the three patients were analyzed in the same manner, and the critical amino acids for IgE-binding were found to be the same as in patient 1 (data not shown).

Immunodominance of the ω-5 Gliadin Epitopes—To determine whether any of the seven epitopes of ω-5 gliadin found in this study were immunodominant, each peptide was synthesized and probed with sera of 15 patients. The results are summarized in Fig. 5. The QQIPQQQ and QQFPQQQ peptides were recognized by 13 of 15 WDEIA patients (87%). The QQSPEQQ and QQSPQQQ peptides were recognized by 12 of 15 patients (80%), and the QQLPQQQ and QQYPQQQ epitopes were recognized by 9 of 15 patients and 8 of 15 patients (60 and

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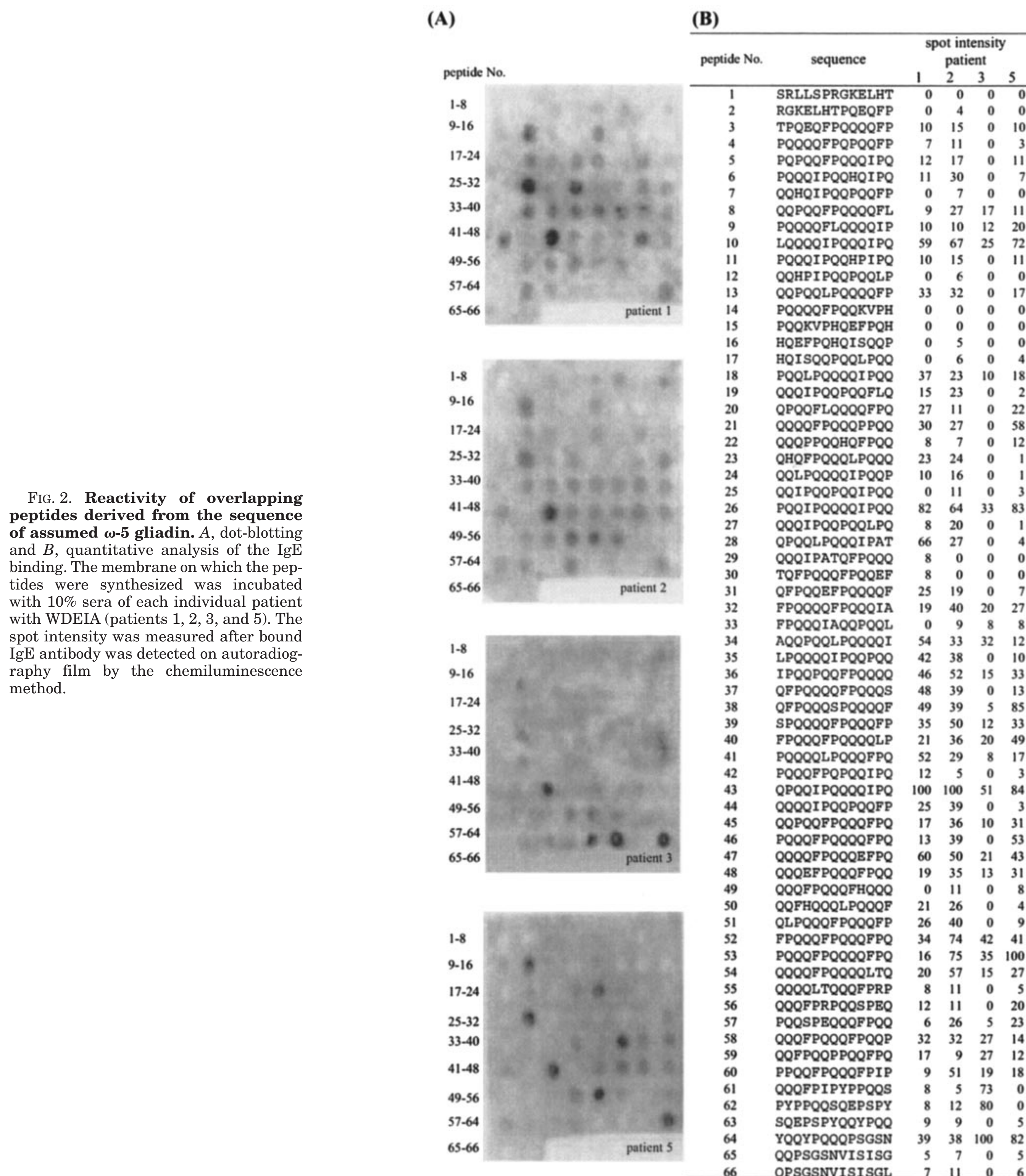

peptide No.	sequence	spot intensity patient			
		1	2	3	5
1	SRLLSPRGKELHT	0	0	0	0
2	RGKELHTPQEQFP	0	4	0	0
3	TPQEQFPQQQQFP	10	15	0	10
4	PQQQQFPQPQQFP	7	11	0	3
5	PQPQQFPQQQIPQ	12	17	0	11
6	PQQQIPQQHQIPQ	11	30	0	7
7	QQHQIPQQPQQFP	0	7	0	0
8	QQPQQFPQQQQFL	9	27	17	11
9	PQQQQFLQQQQIP	10	10	12	20
10	LQQQQIPQQQIPQ	59	67	25	72
11	PQQQIPQQHPIPQ	10	15	0	11
12	QQHPIPQQPQQLP	0	6	0	0
13	QQPQQLPQQQQFP	33	32	0	17
14	PQQQQFPQQKVPH	0	0	0	0
15	PQQKVPHQEFPQH	0	0	0	0
16	HQEFPQHQISQQP	0	5	0	0
17	HQISQQPQQLPQQ	0	6	0	4
18	PQQLPQQQQIPQQ	37	23	10	18
19	QQQIPQQPQQFLQ	15	23	0	2
20	QPQQFLQQQQFPQ	27	11	0	22
21	QQQQFPQQQPPQQ	30	27	0	58
22	QQQPPQQHQFPQQ	8	7	0	12
23	QHQFPQQQLPQQQ	23	24	0	1
24	QQLPQQQQIPQQP	10	16	0	1
25	QQIPQQPQQIPQQ	0	11	0	3
26	PQQIPQQQQIPQQ	82	64	33	83
27	QQQIPQQPQQLPQ	8	20	0	1
28	QPQQLPQQQIPAT	66	27	0	4
29	QQQIPATQFPQQQ	8	0	0	0
30	TQFPQQQFPQQEF	8	0	0	0
31	QFPQQEFPQQQQF	25	19	0	7
32	FPQQQQFPQQQIA	19	40	20	27
33	FPQQQIAQQPQQL	0	9	8	8
34	AQQPQQLPQQQQI	54	33	32	12
35	LPQQQQIPQQPQQ	42	38	0	10
36	IPQQPQQFPQQQQ	46	52	15	33
37	QFPQQQQFPQQQS	48	39	0	13
38	QFPQQQSPQQQQF	49	39	5	85
39	SPQQQQFPQQQFP	35	50	12	33
40	FPQQQFPQQQQLP	21	36	20	49
41	PQQQQLPQQQFPQ	52	29	8	17
42	PQQQFPQPQQIPQ	12	5	0	3
43	QPQQIPQQQQIPQ	100	100	51	84
44	QQQQIPQQPQQFP	25	39	0	3
45	QQPQQFPQQQFPQ	17	36	10	31
46	PQQQFPQQQQFPQ	13	39	0	53
47	QQQQFPQQQEFPQ	60	50	21	43
48	QQQEFPQQQFPQQ	19	35	13	31
49	QQQFPQQQFHQQQ	0	11	0	8
50	QQFHQQQLPQQQF	21	26	0	4
51	QLPQQQFPQQQFP	26	40	0	9
52	FPQQQFPQQQFPQ	34	74	42	41
53	PQQQFPQQQQFPQ	16	75	35	100
54	QQQQFPQQQQLTQ	20	57	15	27
55	QQQQLTQQQFPRP	8	11	0	5
56	QQQFPRPQQSPEQ	12	11	0	20
57	PQQSPEQQQFPQQ	6	26	5	23
58	QQQFPQQQFPQQP	32	32	27	14
59	QQFPQQPPQQFPQ	17	9	27	12
60	PPQQFPQQQFPIP	9	51	19	18
61	QQQFPIPYPPQQS	8	5	73	0
62	PYPPQQSQEPSPY	8	12	80	0
63	SQEPSPYQQYPQQ	9	9	0	5
64	YQQYPQQQPSGSN	39	38	100	82
65	QQPSGSNVISISG	5	7	0	5
66	QPSGSNVISISGL	7	11	0	6

FIG. 2. **Reactivity of overlapping peptides derived from the sequence of assumed ω-5 gliadin.** *A*, dot-blotting and *B*, quantitative analysis of the IgE binding. The membrane on which the peptides were synthesized was incubated with 10% sera of each individual patient with WDEIA (patients 1, 2, 3, and 5). The spot intensity was measured after bound IgE antibody was detected on autoradiography film by the chemiluminescence method.

53%), respectively. These data indicate that the QQIPQQQ, QQFPQQQ, QQSPQQQ, and QQSPEQQ epitopes are immunodominant and that the IgE antibodies of the patients probably cross-react to these epitopes. The PYPP epitope was unique for patient 3. Patients 11 and 13 had no IgE antibody bound to these tested epitopes, although their sera had IgE bound to fast ω-gliadin (Table I).

DISCUSSION

The water/salt-insoluble wheat proteins, termed gluten, have been considered to be causative allergens for WDEIA. Gluten is composed of two classes of proteins, the 70% ethanol-soluble gliadins (α-, β-, γ-, and ω-gliadins) and the 70% ethanol-insoluble glutenins (HMW and LMW glutenins). Among these proteins, fast ω-gliadin, especially ω-5 gliadin, is considered to be a major allergen for WDEIA (20, 26, 27). In this study, we determined IgE binding against a panel of purified gluten proteins by using sera obtained from 15 patients defined as having WDEIA by a challenge test and found that ~80% of the patients reacted to fast ω-gliadin most strongly, confirming that the fast ω-gliadin is a predominant allergen for WDEIA (Table I).

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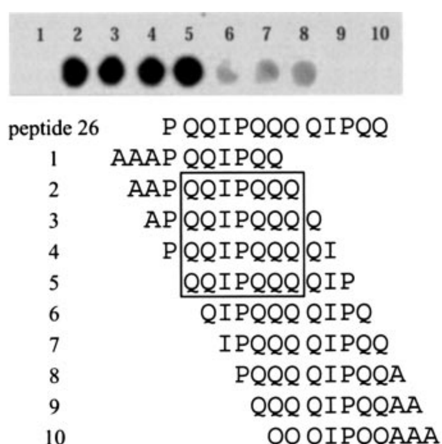

FIG. 3. **Identification of a core IgE-binding epitope of ω-5 gliadin.** The data obtained with PQQIPQQQQIPQQ (peptide 26 in Fig. 2) were shown representatively. The overlapping peptides 10 amino acids in length offset by one were synthesized and probed with 10% sera of patients. The appropriate alanine residues were added to both ends of the peptide to conform to the length of the peptides.

TABLE II
IgE-binding epitope sequences for patients with WDEIA

Patients			
1	2	3	5
QQIPQQQ	QQIPQQQ	QQIPQQQ	QQIPQQQ
QQLPQQQ	QQLPQQQ	QQLPQQQ	
QQFPQQQ	QQFPQQQ	QQFPQQQ	QQFPQQQ
QQSPEQQ	QQSPEQQ	QQSPEQQ	QQSPEQQ
QQSPQQQ	QQSPQQQ	QQSPQQQ	QQSPQQQ
QQYPQQQ	QQYPQQQ	QQYPQQQ	QQYPQQQ
		PYPP	

We then analyzed the epitope sequences by means of peptide array of assumed ω-5 gliadin with the sera of four. The epitope sequences of ω-5 gliadin were found to be QQ*X*PQQQ (*X* = I, F, S, L, or Y), QQSPEQQ, and PYPP (Table II), four of which, QQ*X*PQQQ (*X* = I, F, or S) and QQSPEQQ, were immunodominant. These epitopes appear frequently in the amino acid sequence of ω-5 gliadin as shown in Fig. 2. This is compatible with the data reported by DuPont *et al.* (23) in which repeats of QQ*X*P, QQQ*X*P, or QQQQ*X*P (*X* = F, I, and L in order of predominance) are characteristic for the ω-5-type gliadins. We further determined that the critical amino acids in the QQIPQQQ and QQFPQQQ epitopes were positions Gln^1, Pro^4, Gln^5, Gln^6, and Gln^7, suggesting the epitope sequence Q*XX*-PQQQ. In addition, it was speculated that the glutamine residue in the epitope sequence could be replaced by glutamic acid without loss of allergenic ability because both QQSPQQQ and QQSPEQQ epitopes were recognized equally. These data suggest that there are many IgE-binding sites in the ω-5 gliadin. This may account for the serious allergic reactions such as shock, which is characteristic of WDEIA, despite a low proportion of ω-5-type gliadins in gluten (24, 31).

2 of the 15 patients with WDEIA (patients 11 and 13) had no specific IgE bound to these epitopes (Fig. 5), although their sera had IgE bound to fast ω-gliadin. These results suggest that the two patients recognize other epitopes in the ω-5 gliadin or that there was nonspecific binding. Interestingly, the sera of these patients in addition to patient 4 reacted to HMW glutenin dominantly among purified water/salt-insoluble wheat proteins (Table I). Taking into consider the relatively low reaction to HMW glutenin in control subjects, these results might suggest that there is another minor subgroup in WDEIA, which reacts to other epitopes in HMW glutenin as a cause of the disease.

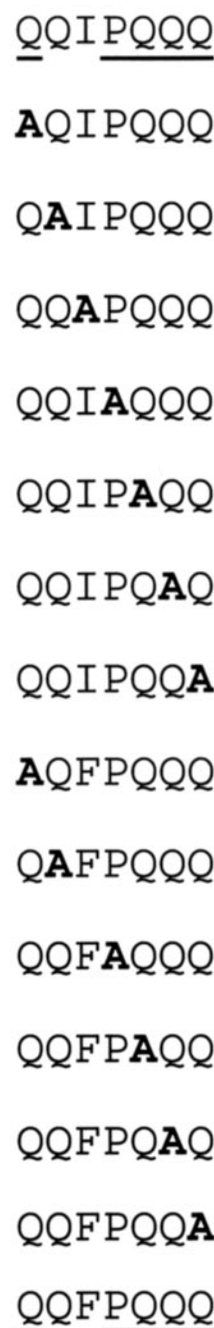

FIG. 4. **Critical amino acids were analyzed in two epitopes (QQIPQQQ and QQFPQQQ) of ω-5 gliadin.** The data shown represent the results for patient 1. Two epitopes were synthesized with an alanine residue substituted for one of the amino acids at each position in the peptide. The membrane was probed with 10% sera of patients. Alanine substitutions are shown in *boldface letter*, and the critical amino acids are *underlined*.

This study also showed that the sera of all 15 patients with WDEIA reacted to slow ω-gliadin, LMW, and HMW glutenin apart from the strong reactivity to fast ω-gliadin (Table I). Previously, we reported (20) that fast ω-gliadin cross-reacts to γ-gliadin and slow ω-gliadin, suggesting high sequence homology in these proteins. Palouso *et al.* (32) also revealed that γ-70 and γ-35 secalins in rye and γ-3 hordein in barley cross-react with ω-5 gliadin (32). This is also supported by amino acid sequences of the several gliadins thus far reported. The amino acid sequence of slow ω-gliadin is homologous to the C-hordein

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epitopes	patients														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
QQIPQQQ	■	■	■	■	■	■	■	■	■	■		■		■	■
QQLPQQQ	■	■	■			■		■		■		■		■	■
QQFPQQQ	■	■	■	■	■	■	■	■	■	■		■		■	■
QQSPEQQ	■	■	■	■	■	■	■		■	■		■		■	■
QQSPQQQ	■	■	■	■	■	■	■		■	■		■		■	■
QQYPQQQ	■	■	■		■	■	■					■		■	
PYPP			■												

FIG. 5. **Overview of the reactivity against the epitopes.** Sera of 15 patients were tested against the epitopes characterized in this study. The *filled box* indicates that sera from patients with WDEIA had specific IgE to the epitope.

of barley and ω-secalins of rye and has $PFPQ_{1-2}PQQ$ repeat motif in repetitive domain (33). The sequence of γ-gliadin also has $PFPQI_{1-2}(PQQ)_{1-2}$ motif that is similar to slow ω-gliadin in the repeat domain (34), and there is a QPYPQQQ sequence in slow ω-gliadin and QSFPQQQ and QPFPQQQ sequences in γ-gliadin. Because these sequences are very similar to the epitopes of ω-5 gliadin and include critical amino acids for IgE binding, it is likely that specific IgE to ω-5 gliadin cross-reacts with γ-gliadin and slow ω-gliadin. In addition, the sera of some patients had specific IgE to α-gliadin, which had been reported as a major allergen for WDEIA by Palosuo *et al.* (26). The α-gliadin has QQLPQEQ, QQQPQEQ, and QPYPQQQ sequences (35); therefore, this may cross-react with ω-5 gliadin as well as γ-gliadin and slow ω-gliadin. Moreover, the IgE-binding epitope of ω-5 gliadin QQLPQQQ was seen in the VolckA3 and LMW21 and QQFPQQQ was seen in the VolckB3, respectively, all of which belong to the LMW glutenins (36), suggesting that ω-5 gliadin also cross-reacted with LMW glutenin. These results might account partly for the diversity of the reactivity in the dot-blot analysis with the sera of the patients.

These IgE-binding epitopes are different from QQQPP previously identified by Tanabe *et al.* (18) as the major allergen for wheat allergy. In this study, we synthesized the peptide SQQQPPF and probed with the sera of the WDEIA patients; however, only a weak IgE-bound signal to this peptide was observed in 10 of 15 patients (67%) (data not shown). The result indicates that the IgE-binding epitope sequences, QQ*X*PQQQ (*X* = I, F, S, L, or Y), QQSPEQQ, and PYPP, are distinct and responsible for WDEIA. These motifs do not exist in the proteins, which are major allergens for bakers' asthma, such as α-amylase inhibitors peroxidase and serpin, supporting the idea that these epitopes are characteristic for WDEIA.

The CAP-RAST system is now widely used for the diagnosis of allergy, whereas as found in this study, some patients with definite WDEIA were negative in CAP-RAST for gluten (Table I), suggesting unreliable sensitivity of the test for the diagnosis of WDEIA. In addition, a number of the patients with atopic dermatitis reacted positively to CAP-RAST for gluten, although the patients did not have an anaphylactic reaction to gluten such as control subjects 31 and 32 in Table I. These findings suggest that the measurement of specific IgE for gluten is not always satisfactory for the screening as well as diagnosis of WDEIA. It is conceivable that the simultaneous measurement of specific IgE antibodies to both fast-ω gliadin and the epitope peptides was more sensitive and specific to diagnose WDEIA than that to gluten. In fact, to diagnose WDEIA, the clinical efficacy of the measurement of specific IgE to ω-5 gliadin has already been reported (26, 27).

Food-dependent exercise-induced anaphylaxis is a life-threatening disease, but the only reliable therapy is strict elimination of allergen from dietary foods. However, it is not easy to eliminate wheat from foodstuff for a long time. Mutated allergen protein immunotherapy or peptide immunotherapy for food allergy was successful in animal models such as peanut anaphylaxis by means of the allergens or peptides lacking the ability to bind to IgE but retaining the ability to activate T-cells (37). The elucidation of the major IgE-binding epitopes on ω-5 gliadin in this study may provide a useful tool for developing hypoallergenic foods as well as a new diagnostic technique and immunotherapy for patients with WDEIA.

REFERENCES

1. Kidd, J. M., Cohen, S. H., Sosman, A. J., and Fink, J. N. (1983) *J. Allergy Clin. Immunol.* **71,** 407–411
2. McNeil, D., and Strauss, R. H. (1988) *Ann. Allergy* **61,** 440–442
3. Martin Munoz, F., Lopez Cazana, J. M., Villas, F., Contreras, J. F., Diaz, J. M., and Ojeda, J. A. (1994) *Allergy* **49,** 314–316
4. Noma, T., Yoshizawa, I., Ogawa, N., Ito, M., Aoki, K., and Kawano, Y. (2001) *Asian Pac. J. Allergy Immunol.* **19,** 283–286
5. Pauls, J. D., and Cross, D. (1998) *J. Allergy Clin. Immunol.* **101,** 853–854
6. Silverstein, S. R., Frommer, D. A., Dobozin, B., and Rosen, P. (1986) *J. Emerg. Med.* **4,** 195–199
7. Dohi, M., Suko, M., Sugiyama, H., Yamashita, N., Tadokoro, K., Juji, F., Okudaira, H., Sano, Y., Ito, K., and Miyamoto, T. (1991) *J. Allergy Clin. Immunol.* **87,** 34–40
8. Mine, Y., and Zhang, J. W. (2002) *Biochem. Biophys. Res. Commun.* **292,** 1070–1074
9. Busse, P. J., Jarvinen, K. M., Vila, L., Beyer, K., and Sampson, H. A. (2002) *Int. Arch. Allergy Immunol.* **129,** 93–96
10. Cocco, R. R., Jarvinen, K. M., Sampson, H. A., and Beyer K. J. (2003) *J. Allergy Clin. Immunol.* **112,** 433–437
11. Xiang, P., Beardslee, T. A., Zeece, M. G., Markwell, J., and Sarath, G. (2002) *Arch. Biochem. Biophys.* **408,** 51–57
12. Rabjohn, P., Helm, E. M., Stanley, J. S., West, C. M., Sampson, H. A., Burks, A. W., and Bannon, G. A. (1999) *J. Clin. Invest.* **103,** 535–542
13. Amano, M., Ogawa, H., Kojima, K., Kamidaira, T., Suetsugu, S., Yoshihama, M., Satoh, T., Samejima, T., and Matsumoto, I. (1998) *Biochem. J.* **330,** 1229–1234
14. Yamashita, H., Nanba, Y., Onishi, M., Kimoto, M., Hiemori, M., and Tsuji, H. (2002) *Biosci. Biotechnol. Biochem.* **66,** 2487–2490
15. Sander, I., Flagge, A., Merget, R., Halder, T. M., Meyer, H. E., and Baur, X. (2001) *J. Allergy Clin. Immunol.* **107,** 907–913
16. Sandiford, C. P., Tatham, A. S., Fido, R., Welch, J. A., Jones, M. G., Tee, R. D., Shewry, P. R., and Newman Taylor, A. J. (1997) *Clin. Exp. Allergy* **27,** 1120–1129
17. Watanabe, M., Tanabe, S., Suzuki, T., Ikezawa, Z., and Arai, S. (1995) *Biosci. Biotechnol. Biochem.* **59,** 1596–1597
18. Tanabe, S., Arai, S., Yanagihara, Y., Mita, H., Takahashi, K., and Watanabe, M. (1996) *Biochem. Biophys. Res. Commun.* **219,** 290–293
19. Maruyama, N., Ichise, K., Katsube, T., Kishimoto, T., Kawase, S., Matsumura, Y., Takeuchi, Y., Sawada, T., and Utsumi, S. (1998) *Eur. J. Biochem.* **255,** 739–745
20. Morita, E., Matsuo, H., Mihara, S., Morimoto, K., Savage, A. W. J., and Tatham, A. S. (2003) *J. Dermatol. Sci.* **33,** 99–104
21. Morita, E., Yamamura, Y., Mihara, S., Kameyoshi, Y., and Yamamoto, S. (2000) *Br. J. Dermatol.* **143,** 1059–1063
22. Morita, E., Kameyoshi, Y., Mihara, S., Hiragun, T., and Yamamoto, S. (2001) *Br. J. Dermatol.* **145,** 182–184
23. DuPont, F. M., Vensel, W. H., Chan, R., and Kasarda, D. D. (2000) *Cereal Chem.* **77,** 607–614
24. Seilmeier, W., Valdez, I., Mendez, E., and Wieser, H. (2001) *Eur. Food Res. Technol.* **212,** 355–363
25. Kasarda, D. D., Autran, J. C., Lew, E. J. L., Nimmo, C. C., and Shewry, P. R. (1983) *Biochim. Biophys. Acta* **747,** 138–150
26. Palosuo, K., Alenius, H., Varjonen, E., Koivuluhta, M., Mikkola, J., Keskinen, H., Kalkkinen, N., and Reunala, T. (1999) *J. Allergy Clin. Immunol.* **103,** 912–917
27. Palosuo, K., Varjonen, E., Kekki, O. M., Klemola, T., Kalkkinen, N., Alenius, H., and Reunala, T. (2001) *J. Allergy Clin. Immunol.* **108,** 634–638
28. Palosuo, K., Varjonen, E., Nurkkala, J., Kalkkinen, N., Harvima, R., Reunala, T., and Alenius, H. J. (2003) *J. Allergy Clin. Immunol.* **111,** 1386–1392
29. Sigurs, N., Hattevig, G., Kjellman, B., Kjellman, N. I., Nilsson, L., and Bjorksten, B. (1994) *J. Allergy Clin. Immunol.* **94,** 757–763
30. Tatham, A. S., and Shewry, P. R. (1985) *J. Cereal Sci.* **3,** 103–113
31. Wieser, H. (2000) *Eur. Food Res. Technol.* **211,** 262–268
32. Palosuo, K., Alenius, H., Varjonen, E., Kalkkinen, N., and Reunala, T. (2001) *Clin. Exp. Allergy* **31,** 466–473
33. Hsia, C. C., and Anderson, O. D. (2001) *Theor. Appl. Genet.* **103,** 37–44
34. Anderson, O. D., Hsia, C. C., and Torres, V. (2001) *Theor. Appl. Genet.* **103,** 323–330
35. Anderson, O. D., and Greene, F. C. (1997) *Theor. Appl. Genet.* **95,** 59–65
36. Cassidy, B. G., Dvorak, J., and Anderson, O. D. (1998) *Theor. Appl. Genet.* **96,** 743–750
37. Nowak-Wegrzyn, A. (2003) *Pediatrics* **111,** 1672–1680

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Identification of the IgE-binding Epitope in ω-5 Gliadin, a Major Allergen in Wheat-dependent Exercise-induced Anaphylaxis
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