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Specific IgE Determination to Epitope Peptides of ω-5 Gliadin and High Molecular Weight Glutenin Subunit Is a Useful Tool for Diagnosis of Wheat-Dependent Exercise-Induced Anaphylaxis

Hiroaki Matsuo, Kunie Kohno, Hiroyuki Niihara, and Eishin Morita

Wheat ω-5 gliadin and a high m.w. glutenin subunit (HMW-glutenin) have been reported as major allergens in wheat-dependent exercise-induced anaphylaxis. A simultaneous detection of specific IgE to epitope sequences of both proteins is considered to be a reliable method for diagnosis of wheat-dependent exercise-induced anaphylaxis. However, the IgE-binding epitope of HMW-glutenin remains unknown. The aim of this study was to determine the IgE-binding epitopes of HMW-glutenin to establish a useful system of identifying patients with wheat-dependent exercise-induced anaphylaxis. For determination of IgE-binding epitopes of HMW-glutenin overlapping peptides were synthesized and reactivities of IgE Abs in the sera of patients to those peptides were analyzed. Three IgE-binding epitopes, QQPGQ, QQPGQGQQ, and QQQSGQGQ, were identified within primary sequence of HMW-glutenin. Epitope peptides, which include IgE-binding sequences of ω-5 gliadin and a HMW-glutenin, were synthesized and peptide-specific IgE Abs were measured by CAP-System fluorescent enzyme immunoassay. Twenty-nine of 30 patients with wheat-dependent exercise-induced anaphylaxis had specific IgE Abs to these epitope peptides. None of the 25 sera from healthy subjects reacted to both epitope peptides. Twenty-five patients with atopic dermatitis who had specific IgE to wheat and/or gluten had very low or nonexistent levels of epitope peptide-specific IgE Abs. These results indicated that measurement of IgE levels specific to epitope peptides of ω-5 gliadin and HMW-glutenin is useful as an in vitro diagnostic method for the assessment of patients with wheat-dependent exercise-induced anaphylaxis. The Journal of Immunology, 2005, 175: 8116–8122.

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**Abbreviations used in this paper:** FDEIA, food-dependent exercise-induced anaphylaxis; WDEIA, wheat-dependent exercise-induced anaphylaxis; HMW-glutenin, high m.w. glutenin subunit; RAST, radioallergosorbent test; FEIA, fluorescent enzyme immunoassay.
of measurement of IgE Abs specific to combined epitope peptides of α-5 gliadin and HMW-glutenin.

Materials and Methods

Subjects
Thirty patients with WDEIA (13 females, 17 males, mean age: 32.3 years, range: 10–75 years, mean total IgE: 987.7 IU/ml, range: 21.9–14,723) with recurrent episodes of anaphylaxis and a positive provocation test after wheat ingestion took part in this study. Twenty-five healthy subjects (10 females, 15 males, mean age: 33.4 years, range: 18–55 years, mean total IgE: 162.2 IU/ml, range: 7.2–500) and 25 patients with atopic dermatitis (11 females, 14 males, mean age: 24.8 years, range: 5–49 years, mean total IgE: 7,931.8 IU/ml, range: 277–21,699) with wheat and/or gluten-specific IgE Abs in their serum served as controls. Detailed interviews were conducted to determine that the healthy subjects and atopic dermatitis patients had experienced no episode of allergic reaction after ingestion of wheat products. Sera were collected from the subjects and stored at −80°C until use. Specific IgE Abs for wheat and gluten in the sera were determined using the CAP-System fluorescent enzyme immunoassay (FEIA) (Pharmacia Diagnostics).

Dot blotting, SDS-PAGE, and Western blotting
Specific IgE Abs in the sera for α-gliadin, β-gliadin, γ-gliadin, α-5 gliadin, α-1,2 gliadin, HMW-glutenin, and a low m.w. glutenin subunit purified from wheat flour were detected, and spot intensities were measured using the method described previously (13). SDS-PAGE was performed with 12.5% acrylamide gel, and fractionated proteins were then visualized by Coomassie brilliant blue staining. For Western blotting, the fractionated protein was electrophoretically transferred to a polyvinylidene difluoride membrane (Immobilon) and blocked with 5% skim milk in TBST (50 mM Tris-buffered saline and 1% Tween 20 (pH 7.4)). The membrane was washed with TBST three times and then probed with 10% patient’s serum. After washing with TBST, the membrane was incubated with HRP-conjugated rat anti-human IgE mAbs (BioSource International). To detect human IgE binding, ECL Plus Western blotting detection reagents (Amersham Biosciences) was used. The resulting light was detected on autoradiography film.

Peptide synthesis on the SPOTs membrane and IgE-binding assay
Based on the amino acid sequence of the HMW-glutenin (GenBank accession no. X12928), the individual peptides were synthesized on a SPOTs membrane (Sigma-Genosys) described previously (13). The SPOTs membrane was blocked overnight at 4°C with blocking buffer (Sigma-Genosys). The membrane was washed with TBST three times for 10 min at room temperature and then incubated overnight at 4°C with 10% patient’s serum. The bound IgE Abs were detected as described above. The SPOTs membrane was used repeatedly after the following procedure. The membrane was washed with water, N,N-dimethylformamide (Wako Pure Chemical), regeneration buffer A (8 M urea and 10% SDS), regeneration buffer B (6 M guanidine hydrochloride), regeneration buffer C (10% (v/v) acetic acid and 50% (v/v) ethanol), and then rinsed with methanol twice for 10 min at room temperature.

IgE-binding inhibition assay of combined epitope peptides

The epitope peptides, KPQQQSPQQQPQQQ (peptide A) and PTPQQSGQQPGQQ (peptide B), were synthesized to a purity of at least 80% (Thermo Electron). Sera from patients with WDEIA were then preincubated with or without synthetic epitope peptide A or B (100 μg/ml) for 1 h at room temperature. Remaining serum IgE binding to each epitope peptide on the SPOTs membrane was then measured as described above.

Table I. Results of dot blot analysis

<table>
<thead>
<tr>
<th>Patients</th>
<th>Total IgE (IU/mL)</th>
<th>Gluen RAST (IU/dL)</th>
<th>Reactivity (spot intensity)</th>
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<tr>
<td></td>
<td></td>
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<td>6</td>
<td>976</td>
<td>5.95</td>
<td>34</td>
</tr>
</tbody>
</table>

<sup>a</sup> α-gliadin, β-gliadin, γ-gliadin, α-5 gliadin, α-1,2 gliadin, HMW-glutenin, γ-gliadin, α-1,2, α-1,2 gliadin; HMW, high M.W. glutenin subunit; LMW, low M.W. glutenin subunit.

Results

Immunoblotting for purified wheat gliadins and glutenins
The sera of 30 patients with WDEIA were analyzed for the reaction to purified α-gliadin, β-gliadin, γ-gliadin, α-1,2 gliadin, α-5 gliadin, a low m.w. glutenin subunit, and HMW-glutenin. The strongest reaction was observed with α-5 gliadin, HMW-glutenin, and α-1,2 gliadin in 23, 6, and 1 of the 30 patients, respectively. Sera of these six patients whose IgE Abs reacted to HMW-glutenin predominantly were used for epitope mapping. Table I shows the result of the dot-blotting analysis of the patients whose IgE Abs in the sera reacted to HMW-glutenin predominantly.

HMW-glutenins are encoded at the Glu-1 loci on chromosomes 1A, 1B, and 1D, and each locus consists of two genes encoding an x-type and a y-type subunit (21). However, common wheat contains three to five subunits because some of the genes are silent (22). Therefore, we determined which type of HMW-glutenin is the major IgE-binding allergen for the six patients by Western blot experiment with purified protein. The IgE Abs in the sera of all patients reacted to both x-type and y-type HMW-glutenin subunits (Fig. 1).

Multiple IgE-binding epitopes in the HMW-glutenin
Because the amino acid sequences of HMW-glutenin x-type subunit and y-type subunit are very similar and the content of x-type subunit is higher than that of y-type in common wheat flour (22), we used the x-type HMW-glutenin for determination of IgE binding epitope.

Several studies of IgE-binding epitopes of wheat gliadins and glutenins have shown that the length of the epitope sequences is <7 aa (13, 23, 24). Therefore, to determine the regions recognized by the serum IgE Abs of patients with WDEIA, overlapping peptides 14 aa long and offset by 7 aa were synthesized using the SPOTs method based on the amino acid sequence of HMW-glutenin (Fig. 2). Individual sera from six patients were used to map IgE-binding regions in the amino acid sequence of HMW-glutenin. Fig. 3 summarizes the IgE-binding peptides of each of the six patients. Several peptides were reacted by rat anti-human IgE mAbs directly as shown in Fig. 3. Therefore, these peptides were neglected. Many IgE-binding peptides were detected; however, most of these positive sequences were overlapped among the
tested patients. From the results of the IgE-bound peptide sequences alignment, we expected that major epitope sequences are QQPGQ for patients 1 and 4, QQPGQGQQ for patient 2, and QQSGGQQ for patients 2–6 (Fig. 3).

**Determination of the core epitope sequence and critical amino acids for IgE binding**

To determine the exact amino acid sequence of IgE-binding regions, deletion peptides were further synthesized with respect to the IgE-bound peptides, peptides 17 and 69, respectively. These peptides were then probed with the sera of six patients with WDEIA. The immunoblotting results of peptides 17 and 69 with the sera of the patients were shown in Fig. 4. The QQPGQ sequence was detected as IgE-binding epitope for the patients 1 and 4, whereas an additional GQQ sequence was necessary for binding of IgE Abs in the serum of patient 2. Five of six patients’ sera (patients 2–6) reacted to the synthetic peptides having QQSGGQQ sequence. The results of immunoblots using synthetic peptides of assumed core epitope sequence confirmed that the core epitope sequences of HMW-glutenin are QQPGQ, QQPGGQQQ, and QQSGGQQ (Fig. 4C).

The critical amino acids for IgE binding in three epitopes, QQPGQ, QQPGGQQQ, and QQSGGQQ, were determined by synthesized peptides with single amino acid substitution at each position, followed by probing with individual sera from six patients. The results of an immunoblot experiment are shown in Fig. 5, and critical amino acid positions for IgE binding are summarized in Table II. In patient 1, no binding of the serum IgE was observed when alanine was substituted at each position of QQPGQ, indicating that entire amino acid sequence is critical for IgE binding. However, in patient 4, the QQPG sequence (positions 1–4) in the QQPGQ sequence was found to be critical. For the epitope QQPGGQQQ in patient 2, positions 1–5 (QQPQQ) and position 7 (Q) were critical for IgE binding. The critical amino acids for IgE binding in the epitope QQSGGQQQ were positions 1 (Q), 3 (S), 4 (G), 5 (Q), and 7 (Q) in patients 2–6.

**Specific IgE to the synthetic combined epitope peptides of α-5 gliadin and HMW-glutenin**

Epitope peptides, KPQQQPQQPQFPQPPQQP (peptide A) and PTPQQSPQPQGQQPGQQ (peptide B), were designed and synthesized. Peptide A was combined with the IgE-binding epitope sequences of α-5 gliadin, QQIPQQQ, QQFPQQQ, and QQSPQQQ, and peptide B was combined with the three epitope peptides of HMW-glutenin identified in this study. To confirm that the IgE-binding ability of the synthetic combined peptide was equal to that of the individual epitope peptides, IgE-binding inhibition experiments were performed. Peptide A inhibited serum IgE binding to QQIPQQQ, QQFPQQQ, and QQSPQQQ, which are individual synthetic epitope peptides of α-5 gliadin. Similarly, combined epitope peptide B inhibited IgE binding to individual epitope peptides of HMW-glutenin (Fig. 6).

Specific IgE levels of peptides A and B in the sera of patients with WDEIA and controls were determined using Pharmacia CAP-System FEIA (Pharmacia Diagnostics). When a cutoff value in CAP-System FEIA is set at 0.35 kUa/L, 29 of 30 (97%) patients with WDEIA had specific Abs to peptide A and/or peptide B (Fig. 7). One of these 29 patients had specific IgE Abs to both peptide A and B. In contrast, only 18 of 30 (60%) and 24 of 30 (80%) patients with WDEIA showed positive reaction to wheat and gluten, respectively, in the CAP-System FEIA. None of the 25 healthy control subjects had IgE Abs specific to wheat, gluten, peptide A, and peptide B.

Sera from patients with atopic dermatitis who had positive CAP-RAST value for wheat and/or gluten but had no episode of anaphylaxis or urticaria after ingestion of wheat products were also analyzed. Four and 10 of 25 patients with atopic dermatitis had positive CAP-RAST to peptide A and peptide B, respectively. However, the RAST values for epitope peptide A and B remained at low levels ranging from 0.35 to 1.19 kUa/L (Fig. 7).

**Discussion**

In this study, we identified three IgE-binding epitopes in primary sequence of wheat HMW-glutenin and developed a new diagnostic tool for the diagnosis of WDEIA. Our findings suggest that IgE-binding epitopes are localized in the primary sequence of HMW-glutenin and that the epitopes can be used for the diagnosis of WDEIA.
tool that can identify patients with WDEIA using the synthetic epitope peptides of α-5 gliadin and HMW-glutenin.

Patients with WDEIA had been divided into two groups by means of reactivity of serum IgE to wheat proteins: one is α-5 gliadin-reacting group (~80% of patients) and the other is HMW-glutenin-reacting group (12, 13). We had already identified the amino acid sequence of QXXPQQQ (X = any amino acid) as a major IgE-binding epitope of α-5 gliadin. However, epitopes of HMW-glutenin have not been identified. In the present study, we analyzed the IgE-binding epitope sequences by means of peptide array of HMW-glutenin with the six patients’ sera. The epitope sequences of HMW-glutenin were QQSGQGQ, QQPGQ, and QQPGQGQQ and different from the IgE epitopes of gliadins and low m.w. glutenin subunits as reported previously (23, 24). We further determined that the critical amino acids in the three epitopes were positions 1–5 (QQPGQ) or 1–4 (QQPG) for QQPGQ, 1–5 (QQPGQ) and 7 (Q) for QQPGQGQQ, and 1 (Q), 3 (S), 4 (G), 5 (Q), and 7 (Q) for QQSGQGQ, suggesting that QQPGQXQX and QXSGQXQ are major epitope sequences of HMW-glutenin. As shown in Fig. 2, the QQPGQXQX and QXSGQXQ sequences are found in HMW-glutenin x-type amino acid sequence at 8 and 37 sites, respectively. These epitopes also appear

![Figure 3](http://www.jimmunol.org/)

**FIGURE 3.** Analysis of IgE reactivity to overlapping peptides by using sera from six patients with WDEIA and alignment of IgE-bound peptide sequences. The assumed epitope regions are boxed. Control: without patients’ sera.
frequently in the amino acid sequence of HMW-glutenin y-type, of which IgE Abs in the sera of patients reacted in the Western blot experiment (Fig. 1). In addition, serum IgE binding to x- and y-type HMW-glutenin, as shown in Fig. 1, was almost completely inhibited by combined epitope peptide B in all patients (data not shown). These results suggest that x- and y-type HMW-glutenin share epitopes, and all important allergenic epitopes in the HMW-glutenins were detected even though overlapping peptides with an offset of 7 aa were used in this study.

The CAP-System FEIA is an accurate and standardized procedure of determination of allergen-specific IgE and now widely used for the diagnosis of food allergy. The use of food-specific IgE concentrations in predicting clinical reactivity has been reported in egg, milk, peanut, and fish allergies (15). The measurement of wheat or gluten-specific IgE is available for diagnosis of WDEIA in the CAP-System FEIA. Wheat gluten contains about 5% of α-5 gliadin and 9% of HMW-glutenin; however, 20% of patients with definite WDEIA have negative CAP-RAST to gluten. In addition, as shown in this study, a number of the patients with atopic dermatitis have positive CAP-RAST scores for gluten, although the patients have no episode of type I allergic reactions after ingestion of wheat products. Therefore, the measurement of gluten-specific IgE is not always satisfactory for diagnosis of WDEIA. We have suggested that the measurement of specific IgE to α-5 gliadin and HMW-glutenin instead of gluten is useful to diagnose WDEIA (13). Recently, recombinant food allergens have been produced and tried to apply for diagnosis in many food allergies (25, 26). Recombinant proteins of α-5 gliadin and HMW-glutenin have not been available in enough quality and quantity. Hence, in this study, we synthesized the peptides containing major IgE-binding epitopes of those proteins and applied them to CAP-System FEIA to determine the specific IgE to those epitope peptides. As we suspected, 97% (29 of 30) of the patients with WDEIA were found to be positive in IgE specific for synthetic epitope peptides of α-5 gliadin and HMW-glutenin, whereas gluten-specific IgE test was positive in only 80% of patients with WDEIA. In addition, specific IgE values in epitope peptide-specific CAP-System FEIA were much higher than that in the gluten-specific test in most patients with WDEIA, indicating higher sensitivity with epitope-specific system. This is due to higher content of epitope peptides used in the CAP-System FEIA. Ten of 25 patients with atopic dermatitis who had no obvious allergic reactions after ingestion of wheat products had positive CAP-RAST (>0.34 kUa/L) for epitope peptides, indicating low specificity of the test. However, when the

![FIGURE 4](image)

**FIGURE 4.** Identification of core epitope sequences of synthetic peptides. Fine mapping data of peptide 17 (A) and peptide 69 (B) were shown in the six patients with WDEIA. C, Reactivity of the patients’ sera to the three identified epitope peptides. The boxed area indicates the core epitope sequence.

![FIGURE 5](image)

**FIGURE 5.** Critical amino acids were analyzed in three epitopes, QQPGQ, QQPGQQGQ, and QQSGQGQ, in HMW-glutenin. The peptides, including epitope sequences, were synthesized with alanine residue substituted for 1 aa at each position based on the sequence of peptide 17 (A) and peptide 69 (B). The boxed area indicates the epitope sequence.

![FIGURE 6](image)

**FIGURE 6.** Inhibition of IgE binding to individual epitope peptides with combined epitope peptides. A, Serum from a patient with specific IgE Abs to three epitope peptides of α-5 gliadin was preincubated with or without peptide A (KPQQQSPQQQFPPQQQQPQQ) for 1 h at room temperature. B, Sera from two patients, patients 2 and 4, were preincubated with or without peptide B (PTSPQQQSGQQGQQPGQQ). Remaining serum IgE binding to individual epitope peptides on the SPOTs membrane was detected chemiluminescently.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Epitope Sequences</th>
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<tr>
<td>1</td>
<td>QQPGQ</td>
</tr>
<tr>
<td>2</td>
<td>QQPGQQGQ</td>
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<tr>
<td>3</td>
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<td>QQPGQQGQ</td>
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<td>6</td>
<td>QQSGQGQ</td>
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The underline indicates critical amino acids for IgE binding.
cutoff value for positive was set at 1.0 kUa/L, sensitivity and specificity of the epitope peptide-specific CAP-RAST were satisfactory because sensitivity and specificity of the test would reach to 97% (29 of 30) and 98% (1 of 50), respectively.

There are several clinical forms of IgE-mediated allergy to wheat such as, baker’s asthma, common food allergy to wheat, WDEIA, and contact urticaria to wheat flour. Proteins in the water/salt-soluble fraction, such as α-aminolevulinic acid, peroxidase, glyceraldehyde-3-phosphate dehydrogenase, serpin, and triose-phosphate isomerase, have been identified to be major allergens in patients with baker’s asthma (27–29). α-Gliadin, γ-gliadin, and ω-5 gliadin in water/salt-insoluble fraction have been identified as allergens in WDEIA (17, 30, 31). However, in patients with classical wheat allergy, the allergens responsible for symptoms have not been defined because serum IgE reacted to both water/salt-soluble and -insoluble proteins (32–36). As just described, it is considered that causative allergens are variable among the clinical forms (37, 38). Recent studies have shown that ω-5 gliadin is a good candidate as a diagnostic tool for not only WDEIA but also immediate allergy to wheat (39–41). These data suggest that specific IgE determination to epitope peptides of ω-5 gliadin may be useful to diagnose immediate type allergy to wheat as well as WDEIA.

WDEIA is a life-threatening disease, but the only reliable therapy is a strict elimination of wheat products from dietary foods. It is not easy to eliminate wheat as wheat flour is used in many kinds of cooking, so patients with wheat allergy desire hypoallergenic wheat products. Immunotherapy using recombinant-mutated allergens was found to be successful for a peanut allergy in an animal model (42). In combination with the major IgE-binding epitopes of ω-5 gliadin, the elucidation of major IgE-binding epitopes on HMW-glutenin in this study may provide a useful tool for developing hypoallergenic foods as well as an immunotherapy for patients with WDEIA.

In conclusion, our results strongly indicate that determination of specific IgE to synthetic epitope peptide of wheat ω-5 gliadin and HMW-glutenin is useful to diagnose WDEIA. Further studies are required to determine the optimal cutoff values for specific IgE Abs to epitope peptides for predicting clinical outcome in patients with WDEIA.

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Disclosures
The authors have no financial conflict of interest.

References


