

## Short communication

# IgE binding to pepsin-digested water soluble and insoluble wheat proteins

**Background:** Positive skin prick tests to wheat are a common finding among atopic patients, but only a minor fraction of these patients show immediate clinical symptoms after wheat ingestion. The aim of this study was to evaluate the allergenicity of wheat proteins after pepsin treatments.

**Methods:** Six patients with positive specific IgE and/or skin prick test to soluble wheat proteins and to gluten were studied. Five of them had no symptoms after wheat ingestion and showed a negative oral challenge test. All sera were analysed with extracts obtained after pepsin hydrolysis during different time periods of water-soluble and insoluble wheat proteins by IgE immunoblotting.

**Results:** A pepsin-sensitive allergen of around 35 kDa was recognized by the five atopic negative wheat oral challenge patients in the undigested water-insoluble extract. A patient showing immediate urticaria after wheat ingestion detected a pepsin-resistant allergen with similar molecular weight. Finally, an inhalation-induced asthma patient recognized water-soluble proteins (of around 14 kDa) that could correspond to the  $\alpha$ -amylase inhibitors.

**Conclusions:** Our immunoblotting method shows better correlation with the clinical symptoms than skin prick test and CAP results. Pepsin resistance of the acetic acid-soluble wheat proteins may be responsible for the immediate symptoms of the patient who presented immediate urticaria.

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Cereal intake has been associated with some diseases, the first of them, celiac disease, was described many years ago (1). More recently, baker's asthma and food allergy have been described as a consequence of handling or eating cereals. Storage proteins account for 10% of total weight of wheat grain and have been classified into four types according to their solubility: water/salt soluble albumins and globulins and water/salt insoluble gliadins and glutenins (2). Some studies have found a relationship between water-soluble cereal proteins and respiratory diseases such as baker's asthma (3, 4). Water-insoluble proteins have been considered responsible for exercise-induced anaphylaxis (5) and food allergy (6). Palosuo et al. (7) identified an  $\omega$ -5 gliadin (Tri a 19) as a relevant allergen in children with immediate allergy to ingested wheat and wheat-dependent exercise-induced anaphylaxis.

Positive skin prick tests and elevated specific-IgE levels in wheat tolerant patients is a common finding. Sampson and Ho (8) reported a low incidence (10%) of positive wheat oral challenge test in patients with high specific-IgE levels. According to Palosuo (7), this fact could be due to the use of antigen extracts containing water-soluble wheat proteins. Another explanation could be the digestive hydrolysis suffered by some wheat allergens. The

influence of pepsin digestion on the allergenicity of wheat proteins has been recently tested on wheat-dependent exercise-induced anaphylaxis patients but not on another kind of sensitized patients (9).

Our study has aimed to evaluate the allergenicity of water-soluble and insoluble wheat proteins after pepsin hydrolysis in patients with positive skin prick tests and specific IgE to wheat and gluten.

## Methods

### Patients

From a total of 110 patients with positive skin prick test and CAP to wheat, six had also positive skin prick test and CAP to gluten. They were studied and classified according to their clinical symptoms:

1. Five patients with atopic dermatitis without symptoms after wheat ingestion.
2. One atopic patient with immediate urticaria after wheat ingestion.
3. One patient who presented asthma episodes when handling wheat flour with negative CAP to gluten was also included as a control to demonstrate the transfer of allergens.

### Allergy tests

Prick tests were performed with a wheat and gluten extract (ALK-Abelló, Madrid, Spain). A wheal of 3 mm and greater than the wheal caused by histamine hydrochloride (10 mg/ml) was regarded as positive. Saline solution was used as negative control. Prick-by-prick tests were performed with 1 g of common wheat flour mixed with 1 ml of distilled water. Detection of total serum IgE and specific-IgE was performed by Pharmacia CAP System FEIA (Uppsala, Sweden).

### Oral challenge test

Double-blinded placebo-controlled tests were performed in seven patients with 1, 5 and 10 g of common wheat flour previously diluted in water and mixed with mashed potatoes and carrots. Doses of wheat flour were administered on the first day in increasing doses every 30 min. For the next 3 days, the patients' intake was 10 g twice a day. The Institutional Review Board of our hospital approved the study protocol.

### Purification of water soluble and insoluble wheat proteins

Five grams of common wheat flour was incubated with 50 ml of 0.5 M NaCl for 1 h at 4°C and centrifuged for 15 min at 4500 *g*. The supernatant containing water-soluble proteins was stored at -20°C. The pellet was washed 3× with 100 ml distilled water. The water-insoluble proteins were extracted with 100 ml of 1% acetic acid by stirring overnight at room temperature. After centrifugation the supernatant containing water-insoluble proteins was stored at -20°C until use.

### Pepsin hydrolysis

Water-soluble extract was incubated in 50 mM HCl and 1 mg/ml pepsin (2200 units/mg; Sigma, St Louis, MO) final concentration at 37°C in a shaking water bath during 5, 30 min, 2 and 48 h, respectively. Water-insoluble protein extract was incubated with pepsin for the same time as the water-soluble proteins. One aliquot contained the undigested extracts.

### SDS-PAGE and immunoblotting

Ten microlitres of the undigested water-soluble and insoluble wheat and pepsin-digested extracts were subjected to electrophoresis in a 12% acrylamide minigel (150 V) under standard SDS-PAGE

conditions and transferred using 50 mM NaCl, 2 mM Tris-HCl (pH 7.4). The different sera were assayed as described (10).

## Results

Table 1 shows the patient's clinical symptoms as well as the *in vivo* and *in vitro* tests. As can be seen, the highest specific-IE values to wheat insoluble and soluble allergens were found in patient 1, but values as high as 18.5 kU/l were found in challenge-test negative patients (patient 3).

### Oral challenge

The wheat-allergic patient suffered urticaria and lip angioedema after 15 min of the second wheat flour dose (5 g). She was treated with corticosteroids and antihistamine drugs. The other five atopic patients remained asymptomatic after challenge.

The patient with asthma by inhaled flour showed no symptoms after the challenge test.

### SDS-PAGE and immunoblotting

Figure 1 shows the SDS-PAGE of the acetic acid-soluble (lane 1), NaCl-soluble (lane 6), and pepsin-digested (lanes 2–5 and 7–10) extracts that were stained with Coomassie blue. A heavily stained band of around 35 kDa present in the water-insoluble extract (lane 1) was readily digested by pepsin disappearing from the gel after 5 min of hydrolysis. In addition, high molecular weight proteins can be observed in pepsin-undigested lanes and low molecular weight pepsin-digested products in pepsin-digested lanes.

Individual IgE immunoblottings are shown in Fig. 2 on the same extracts as in the SDS-PAGE. Immunoblotting A belongs to the cereal-induced urticaria patient (patient 1). This patient detected a protein of around 35 kDa in the undigested water-insoluble extract. After pepsin hydrolysis, the serum recognized peptides of around 30 and 25 kDa even after 48 h of the pepsin hydrolysis. In lane 6 (undigested extract) the patient 1 recognized several

Table 1. Clinical data of patients

| Patient no. | Age/sex | Symptom | Prick test |        | Prick-by-prick test | CAP test (kU/l) |        | Total IgE (kU/l) | Oral challenge reaction  |
|-------------|---------|---------|------------|--------|---------------------|-----------------|--------|------------------|--------------------------|
|             |         |         | Wheat      | Gluten |                     | Wheat           | Gluten |                  |                          |
| 1           | 1/F     | AD      | +          | +      | +                   | 25.6            | 27.3   | 165              | Urticaria/lip angioedema |
| 2           | 16/M    | AD/R    | +          | +      | +                   | 2.37            | 2.04   | 758              | Negative                 |
| 3           | 12/M    | AD/R/A  | +          | +      | +                   | 18.5            | 11.3   | 2000             | Negative                 |
| 4           | 5/M     | AD/R    | +          | +      | –                   | 0.49            | 0.65   | 232              | Negative                 |
| 5           | 7/M     | AD/R    | +          | +      | +                   | 8.48            | 12.4   | 356              | Negative                 |
| 6           | 1/M     | AD      | +          | +      | –                   | 1.67            | 1.24   | 11               | Negative                 |
| 7           | 18/F    | AEC     | +          | +      | –                   | 11.6            | 6.18   | 445              | Negative                 |
| 8           | 20/M    | AC      | +          | +      | +                   | 16.4            | <0.35  | 522              | Negative                 |

AD, atopic dermatitis; R, allergic rhinitis; A, asthma; AEC, exercise-induced anaphylaxis; AC, asthma by flour cereals inhalation.

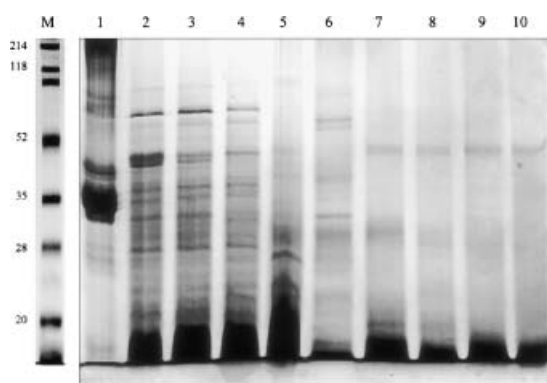


Figure 1. SDS-PAGE (12% acrylamide). Lane M, molecular weight markers; lanes 1–5, wheat water-insoluble proteins; lanes 6–10, wheat proteins extracted in 0.5 M NaCl; lanes 1 and 6, undigested extract; lanes 2 and 7, 5 min of pepsin digestion; lanes 3 and 8, 30 min; lanes 4 and 9, 2 h; lanes 5 and 10, 48 h of pepsin digestion.

pepsin-sensitive proteins of molecular weight between 90 and 15 kDa in the wheat water-soluble extract, which were highly pepsin-sensitive.

The asymptomatic atopic dermatitis patients showed only a weak detection of allergens with a molecular weight around 35 kDa as described in Fig. 2B. These allergens were highly pepsin-sensitive. Finally, Fig. 2C corresponds to a cereal flour-induced asthma (patient no. 7). This subject detected a low molecular weight (around 14 kDa) pepsin-sensitive proteins in the wheat water-soluble extract. The molecular weight of these proteins are similar to that described for the  $\alpha$ -amylase inhibitors (11).

## Discussion

In this study, we aimed to verify if pepsin hydrolysis could influence the allergenicity from wheat proteins. Five of the atopic patients detected by immunoblotting

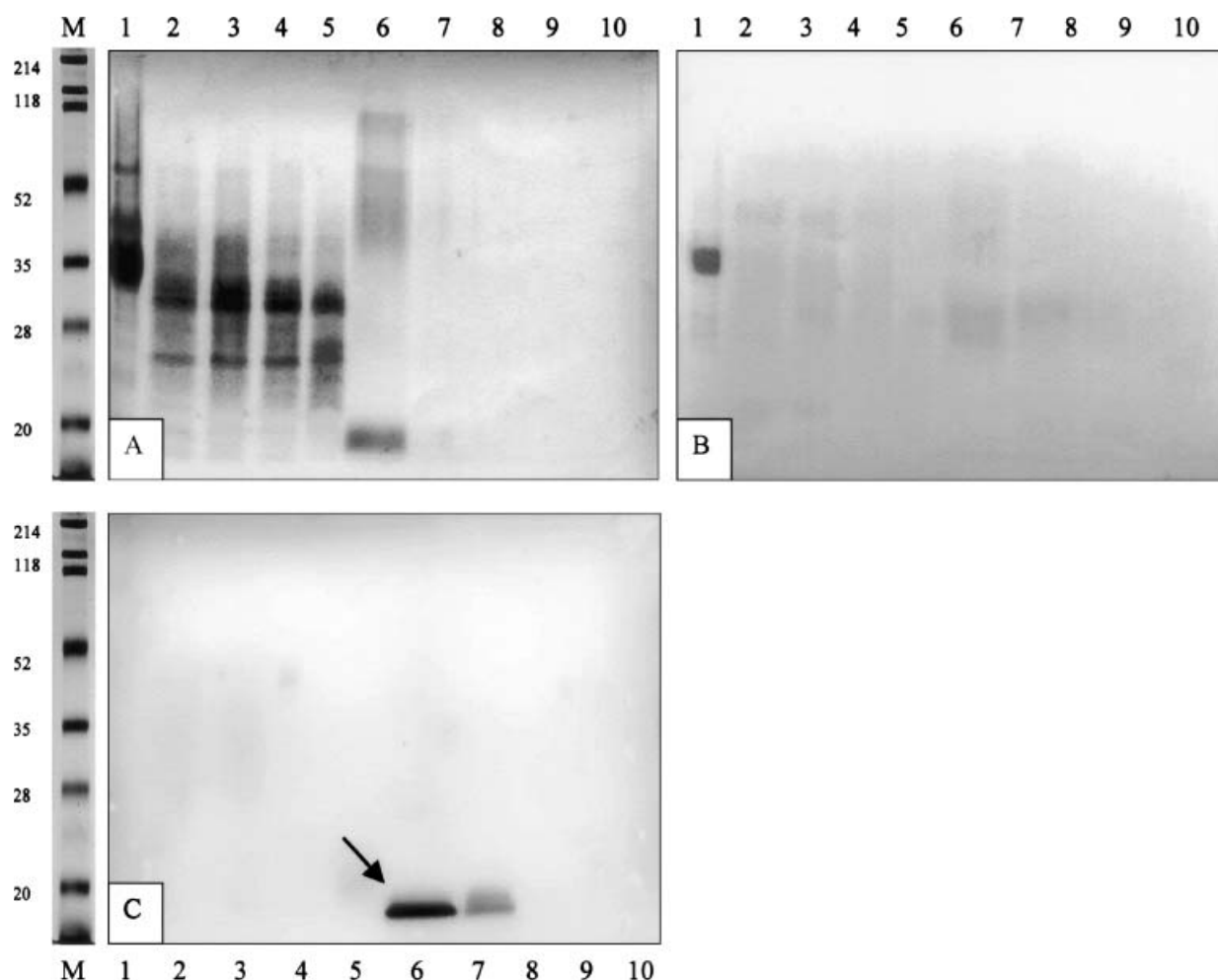


Figure 2. IgE immunoblotting with patient's sera. Antigens are in the same order than in the SDS-PAGE. Arrow in C marks the detection of an allergen of low molecular weight. The following sera were used: the cereal-induced urticaria in 2A (patient no. 1), a negative challenge atopic dermatitis in 2B (patient no. 2) and an asthma by inhaled flour in 2C (patient no. 7). Number of the patients correspond with Table 1.

pepsin-sensitive faint stained bands of around 35 kDa in the undigested water-insoluble extracts. In contrast, the immunoblotting of the patient with cereal-induced urticaria detected a 35 kDa in the undigested water-insoluble extract, and showed in the pepsin-digestion extracts a reduction in the molecular weight suggesting a partial hydrolysis induced by pepsin. This protein had a molecular weight similar to an  $\omega$ -5 gliadin fragment (Tri a 19). After 48-h pepsin-digestion the serum still recognized peptides in the extract. This suggested that IgE epitopes were highly pepsin-resistant. However, this patient also detected a 90 kDa and a low molecular weight stained band in the water-soluble undigested

extract, which was very pepsin-sensitive. Armentia et al. (12) reported that the water-soluble proteins could be responsible for the immediate clinical symptoms after cereal ingestion although this group did not test the pepsin sensitivity of these allergens. Our results clearly demonstrated the different sensitivity to pepsin of water-soluble and insoluble allergens.

As a conclusion, our immunoblotting method showed a better correlation with the clinical symptoms than skin prick test and CAP results. Pepsin resistance of the acetic acid-soluble wheat proteins may be responsible for the symptoms of patients who present immediate urticaria.

## References

1. GEE S. On the coeliac disease. *St Bart Hosp Rep* 1888;**24**:17–20.
2. OSBORNE TB. The proteins of the wheat kernel. Publication number 84. Washington, DC: Carnegie Institution of Washington, 1907.
3. SANCHEZ-MONGE R, GOMEZ I, BARBER D, LOPEZ C, ARMENTIA A, SALCEDO G. Wheat and barley allergens associated with baker's asthma. Glycosylated sub-unit of the alpha-amylase inhibitor family have enhanced IgE-binding capacity. *Biochem J* 1992;**281**:401–405.
4. JAMES JM, SIXBEY JP, HELM RM, BANNON GA, BURKS AW. Wheat alpha-amylase inhibitor: a second route of allergic sensitization. *J Allergy Clin Immunol* 1997;**99**:239–244.
5. PALOSUO K, ALENUS H, VARJONEN E, KALKKINEN N, REUNALA T. Rye  $\gamma$ -70 and  $\gamma$ -35 secalins and barley  $\gamma$ -3 hordein cross react with  $\omega$ -5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. *Clin Exp Allergy* 2001;**31**:466–473.
6. VARJONEN E, VAINIO E, KALIMO K. Antigliadin IgE indicator of wheat allergy in atopic dermatitis. *Allergy* 2000;**55**:386–391.
7. PALOSUO K, VARJONEN E, KKKI OM, KLEMOLA T, KALKKINEN N, REUNALA T. Wheat  $\omega$ -5-gliadin is a major allergen in children with immediate allergy to ingested wheat. *J Allergy Clin Immunol* 2001;**108**:634–638.
8. SAMPSON HA, HO DG. Relationship between food-specific IgE concentration and the risk of positive food challenges in children and adolescent. *J Allergy Clin Immunol* 1997;**100**:444–451.
9. PALOSUO K, VARJONEN E, NURKKALA J, KALKKINEN N, HARVIMA R, REUNALA T et al. Transglutaminase-mediated cross-linking of a peptic fraction of omega-5 gliadin enhances IgE reactivity in wheat-dependent, exercise-induced anaphylaxis. *J Allergy Clin Immunol* 2003;**111**:1386–1392.
10. MONEO I, CABALLERO ML, GÓMEZ F, ORTEGA E, ALONSO MJ. Isolation and characterization of a major allergen from the fish parasite *Anisakis simplex*. *J Allergy Clin Immunol* 2000;**106**:177–182.
11. GOMEZ L, MARTÍN E, HERNANDEZ D, SANCHEZ-MONGE R, BARBER D, DEL POZO V et al. Members of the alpha-amylase inhibitors family from wheat endosperm are major allergens associated with baker's asthma. *FEBS Lett* 1990; **261**:85–88.
12. ARMENTIA A, RODRIGUEZ R, CALLEJO A, MARTÍN-ESTEBAN M, MARTÍN-SANTOS J-M, SALCEDO G et al. Allergy after ingestion or inhalation of cereals involves similar allergens in different ages. *Clin Exp Allergy* 2002;**32**:1216–1222.