

Original article

IgE antibodies to ω -5 gliadin in children with wheat-induced anaphylaxis

Background: Wheat can cause severe immunoglobulin E (IgE)-mediated systemic reactions including anaphylaxis but knowledge on relevant wheat allergens at the molecular level is scanty.

Methods: Seven children (aged from 6 months to 13 years) experiencing from 2 to 10 anaphylactic reactions in a year after eating food-containing wheat were examined. Purified ω -5 gliadin was used as an allergen in IgE enzyme-linked immunosorbent assay (ELISA) and in skin prick testing (SPT). Wheat CAP radioallergosorbent test (RAST) and SPT were also examined.

Results: All seven anaphylactic children, but none of 15 control subjects had IgE antibodies to ω -5 gliadin in ELISA. Five of the six tested anaphylactic children showed positive SPT to ω -5 and crude gliadin, and all seven had positive wheat CAP RAST and SPT. One child was challenged with wheat, which caused anaphylaxis. After adherence to a wheat-free diet four children remained symptomless and three experienced one to two anaphylactic reactions.

Conclusion: The present results show that wheat ω -5 gliadin is a major sensitizing allergen in children with wheat-induced anaphylaxis. They also suggest that ω -5 gliadin IgE ELISA could be used as a diagnostic test for this severe allergy.

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Food allergies affect as many as 6% of young children and mostly present with atopic dermatitis or gastrointestinal symptoms (1). Sensitization to ingested allergens can also lead to severe immunoglobulin E (IgE)-mediated systemic reactions including anaphylaxis (2). Cow's milk, egg, cereals, peanut, soy, fish and shellfish are the most common food allergens (1–3). Peanut has recently received much attention as an important cause of anaphylaxis and its major allergens are well-characterized (4). In contrast, wheat is rarely reported to have caused anaphylaxis in children and the knowledge of ingested cereal allergens at the molecular level is scanty (5–7). Previously, we showed that wheat ω -5 gliadin is a major allergen in adults with food-dependent, exercise-induced anaphylaxis (8). Recently, we found that this water/salt-insoluble allergen is also a potentially important sensitizer in atopic children who react with urticaria or other immediate symptoms to oral wheat challenge (9).

In the present study, we investigated seven children experiencing anaphylactic reactions after ingesting wheat-

containing food and show that they all had IgE antibodies against wheat ω -5 gliadin.

Material and methods

Patients

Seven children (five boys, two girls) with an anaphylactic reaction after ingesting wheat-containing food were examined in the Department of Pediatrics, Siriraj Hospital in Bangkok (Table 1). The study was retrospective and the children were examined from 2 weeks to 8 months after the last anaphylactic reaction. This was defined as angioedema on the face, severe bronchospasm and collapse. Vomitus and generalized urticaria were also common symptoms during the attack. All children had experienced from 2 to 10 anaphylactic reactions in a year. For example, one child (patient number 4, Table 1) ate at the age of 7 months a 1 cm long wheat rusk immediately after which he developed swelling in the face, dyspnea and cyanosis. In the emergency room he was resuscitated and his condition started to improve after the third dose of epinephrine. Four of the seven anaphylactic children were atopic; one

Table 1. Clinical findings in seven children with wheat-induced anaphylaxis

Patient sex/age*	Atopy	Causative food	Attacks in a year	Systemic symptoms	Treatment with epinephrine
1. M/13 (14) years	—	Bun, noodle	3	ANA, URT	+
2. F/6 months (4 years)	SPT+	Cake, macaroni	3	ANA	+
3. M/4 (8) years	—	Pizza, bun	3	ANA, URT	+
4. M/7 (20) months	—	Bread, noodle	4	ANA, URT	+
5. F/3 (14) years	SPT+	Bread	2	ANA	+
6. M/7 (24) months	AE	Bread	2	ANA, URT	+
7. M/7 (12) years	SPT+	Cracker	10	ANA, URT	+

* Onset of symptoms, in parenthesis age at diagnosis.

ANA, anaphylaxis (angioedema on the lips and face, bronchospasm and collapse); URT, generalized urticaria; AE, atopic eczema/dermatitis syndrome; SPT+, skin prick test-positive (patient number 2, egg, fish, shrimp, peanut, house-dust mite; patient number 5 cockroach; patient number 7 egg).

child had atopic eczema/dermatitis syndrome and three had positive skin prick test (SPT) to common inhalant or food allergens (Table 1). Blood was drawn and serum stored at -20°C for IgE enzyme-linked immunosorbent assay (ELISA) and radioallergosorbent test (RAST) examinations. Informed consent was obtained from the parents.

Control sera were obtained from nine Thai children (mean age 8.4 years, eight examined for systemic illnesses) and six young adults (mean age 25 years) who had no history of wheat allergy.

ω -5-Gliadin IgE ELISA and skin prick tests

Wheat ω -5 gliadin was extracted from wheat grains (*Triticum aestivum*) and purified with reversed phase chromatography as previously described (8). For IgE ELISA microtiter plates (100 μl /well) were coated with ω -5 gliadin (2 $\mu\text{g}/\text{ml}$), then 100 μl of patient or control serum (diluted 1 : 10) was added followed by biotinylated goat antihuman IgE (Vector, Burlingame, CA, USA; diluted 1 : 1000), streptavidin-conjugated alkaline phosphatase (Zymed, San Francisco, CA, USA; diluted 1 : 3000) and color substrate (Bio-Rad, Hercules, CA, USA). The color formed was read at 405 nm with an automated ELISA reader (Titertek Multiscan, Eflab, Turku, Finland) as previously described (9). All seven sera from the anaphylactic children were examined in the same run which included a positive and negative control. The CAP RAST (Pharmacia, Uppsala, Sweden) was used to measure IgE antibody levels to crude wheat.

The SPTs were performed with crude wheat [1 : 10 (w/v), 0.9% NaCl], crude gliadin (1 mg/ml) and with purified ω -5 gliadin

(50 $\mu\text{g}/\text{ml}$) as previously described (8). Both gliadins were dissolved in 20% ethanol-phosphate-buffered saline (PBS). Histamine dihydrochloride (10 mg/ml; ALK, Copenhagen, Denmark) served as a positive and saline as a negative control.

Results

All seven children with anaphylaxis showed in ELISA IgE antibodies to ω -5 gliadin (Table 2, Fig. 1). Five of the six tested children showed a positive SPT to crude and ω -5 gliadin, and all seven children had positive wheat RAST and SPT (Table 2). All 15 controls were negative in ω -5 gliadin IgE ELISA (Fig. 1) but one had positive wheat RAST (1.8 kU/l).

Open egg and wheat challenges were performed in one child (patient number 2, Table 1). A challenge with 4 g of egg white was negative. The wheat challenge was started by rubbing the patient's lips with pure spaghetti. Thereafter, the child ate 0.5 g and then 2 g of spaghetti. 30 min later she developed anaphylactic reaction (abdominal pain, vomiting, generalized urticaria, severe bronchospasm and hypotension), which was successfully treated with epinephrine.

All seven children with anaphylaxis adhered to a wheat-free diet. The parents received written instructions

Table 2. Skin prick test, RAST and IgE ELISA results in seven children with wheat-induced anaphylaxis

Patient	Skin prick tests*					
	Histamine (10 mg/ml)	Wheat (1 : 10, w/v)	Gliadin (1 mg/ml)	ω -5 Gliadin (50 $\mu\text{g}/\text{ml}$)	Wheat RAST (KU/l)	ω -5 Gliadin IgE ELISA†
1	10	10	ND	ND	0.9	0.45
2‡	6	16	4	3	>100	3.36
3	5	5	2	0	0.50	1.71
4	4	12	15	7	35	1.86
5	7	7	5	9	2.14	1.31
6	4	10	6	3	10.4	0.31
7	5	5	9	3	1.51	0.36

* Wheal diameter in mm.

† 2 $\mu\text{g}/\text{ml}$; absorbance units at 405 nm.

‡ Oral challenge with wheat caused anaphylaxis.

ND, not done; RAST, radioallergosorbent test; IgE, immunoglobulin E; ELISA, enzyme-linked immunosorbent assay.

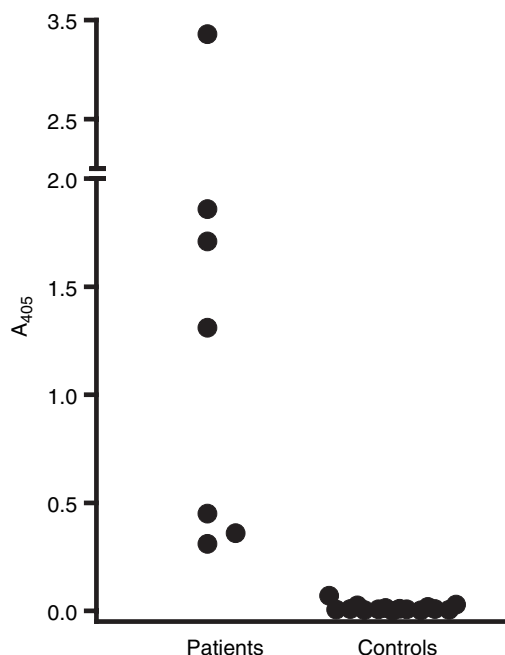


Figure 1. Immunoglobulin E (IgE) antibody levels to ω -5 gliadin in enzyme-linked immunosorbent assay (ELISA) in seven children with wheat-induced anaphylaxis and in 15 control subjects.

on common foods containing wheat and they were advised to carefully check food labeling. During a follow up on a wheat-free diet (from 30 to 47 months) four children remained symptomless. Three children experienced 1–2 anaphylactic reactions because of accidental ingestion of wheat-contaminated food or intentional bread ingestion to establish whether wheat allergy still exists.

Discussion

The results of this study show that all seven children suspected for anaphylaxis from wheat-containing food had IgE antibodies to ω -5 gliadin in ELISA. In addition, five of the six children tested had a positive SPT to ω -5 gliadin further supporting that this water/salt-insoluble gliadin is a major allergen in the children with wheat-induced anaphylaxis. A previous immunoblotting study in seven Japanese children with wheat-induced anaphylaxis showed IgE binding to water/salt-insoluble 16, 38 and 70 kDa wheat proteins (6). These IgE-binding proteins were not characterized but it is quite possible that the 70 kDa allergen could correspond to ω -5 gliadin (8). Water/salt-soluble albumins and globulins appear to be the most important wheat allergens contributing to Baker's asthma (10) whereas information regarding wheat proteins responsible for allergic reactions after ingestion is limited (7, 11, 12). A few studies have

presented evidence that water/salt-insoluble gliadins seem to be important IgE-binding proteins in wheat-allergic children (13, 14). A recent study using highly purified wheat protein fractions in immunoblotting and RAST confirmed that gliadins bind frequently IgE from the sera of wheat-allergic patients (15). The same study showed also that 48% of the 28 wheat-allergic children and adults had IgE antibodies against ω -gliadins. This finding in agreement with the present and our previous study showing that ω -5 gliadin is a major IgE-binding allergen in children with wheat-induced anaphylaxis or other immediate symptoms (9).

There is increasing evidence that wheat is a very important allergen causing food-dependent, exercise-induced anaphylaxis (8, 16–18). The finding that wheat gliadins and especially ω -5 gliadin, triggers production of IgE antibodies both in adults with exercise-induced anaphylaxis (8) and in children with wheat-induced anaphylaxis, as shown in the present study, is of interest. Although both conditions represent allergies to ingested wheat, adults experience recurrent episodes of anaphylaxis only during exercise. We tried to link the anaphylactic reactions in the present children to physical activity by questioning carefully the parents but did not find any evidence for this. Moreover, the youngest children in the present series did not even walk when they experienced the first episode of anaphylaxis. It seems, therefore, that the present children do not need exercise or other triggering factors besides wheat ingestion to develop anaphylaxis. Bread, bun, noodles, pizza and macaroni were the foods causing the anaphylactic reactions in the present children. After ingestion of bread and other wheat products water/salt-insoluble gliadins pass the stomach to small bowel. The IgE-binding ability of ω -5 gliadin is retained after pepsin and trypsin digestion *in vitro* (19). The digested gliadin peptides with IgE-binding epitopes could, therefore, penetrate or be actively transported like other food allergens through the intestinal mucosa and cause anaphylaxis in sensitized children (20–22).

Oral food challenges carry the risk of provoking serious, even life-threatening reactions in children with immediate hypersensitivity (23). In the present study, we observed an anaphylactic wheat challenge reaction in one child. In our previous study, oral wheat challenge caused anaphylaxis in one child although she had no previous history of wheat-induced anaphylaxis (9). Instead of performing potentially dangerous oral wheat challenges, measurement of IgE antibodies to ω -5 gliadin by IgE ELISA seems to be a good method to confirm the diagnosis. The ω -5 gliadin IgE ELISA was positive in all seven children with wheat-induced anaphylaxis but negative in all 15 control subjects. The specificity of ω -5 gliadin IgE ELISA cannot be evaluated in the present study because of the small number ($n = 7$) of children with wheat anaphylaxis. However, in our previous study (9) where outcome was positive or negative wheat

challenge, the sensitivity of the ω -5 gliadin IgE ELISA for detecting immediate allergy to ingested wheat was 84% and the specificity, 100%. The corresponding values for wheat RAST were 95 and 67%.

After diagnosis all children adhered to wheat-free diet and as in our previous study in adults with wheat-dependent, exercise-induced anaphylaxis (8), this markedly reduced recurrences of anaphylaxis. Mistakes following a wheat-free diet can occur especially when eating outside the home. Children with wheat-induced anaphylaxis, like other children at high risk for food-induced anaphylaxis, should receive epinephrine autoinjector for emergency treatment (24). A follow up study is needed to show whether children with wheat-induced

anaphylaxis can outgrow their allergy like children who are allergic to cow's milk (25).

In conclusion, ω -5 gliadin was shown to be a major allergen in the seven children with wheat-induced anaphylaxis.

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