

Short communication

Antigliadin IgE – indicator of wheat allergy in atopic dermatitis

Background: Cereal grains are recognized as the cause of adverse reactions in some patients exposed to grain or flour by either inhalation or ingestion. Cereal-related diseases, such as celiac disease and baker's asthma, have been well studied and the causative cereal proteins have been characterized. Although cereals form an essential part of daily nutrition, the allergenic proteins causing symptoms on ingestion in atopic dermatitis (AD) have remained obscure. In this study, we have investigated the allergenic fraction of wheat in AD.

Methods: Skin prick tests (SPT) with a NaCl wheat suspension and the ethanol-soluble wheat gliadin were performed on 18 wheat-challenge-positive or -negative children with AD, six adult AD patients with suspected cereal allergy, and one adult with wheat-dependent exercise-induced urticaria/anaphylaxis.

Serum total IgE and specific IgE-antibody levels to wheat and gluten were measured with the radioallergosorbent test (RAST) simultaneously. In addition serum samples of all 25 patients were analyzed by IgE immunoblotting with the ethanol-soluble wheat-protein extract.

Results: Thirteen of the AD children were wheat-challenge-positive, 11/12 of them appeared to be positive with gliadin SPT, and all had an elevated gluten RAST value. Those challenge-negative were negative with both gliadin SPT and gluten RAST. Positive wheat SPT and RAST alone were not associated with positive challenges. Four of the adult patients responded to a cereal-free diet, although only two of them appeared to be positive with gliadin SPT and gluten RAST. A broad and intensive staining of gliadin peptides in IgE-immunoblotting studies was seen in challenge-positive children with positive gliadin SPT and/or gluten RAST. Besides staining of peptides in the main gliadin area of 30–46 kDa, a characteristic finding was the staining of small, <14-kDa proteins with sera of challenge- and gliadin-SPT-positive patients.

Conclusions: We found that wheat-allergic AD patients have IgE antibodies against gliadin that can be detected by both SPT and the sensitive immunoblotting method. This suggests that gliadin peptides are important allergens, and ingestion of wheat causes symptoms of AD. A broad and intensive IgE staining was seen of gliadin peptides against both the previously characterized peptides in the main gliadin area and small, previously uncharacterized peptides of less than 14 kDa. The gliadin SPT and gluten RAST are good screening methods. Further characterization of the IgE-stained gliadin proteins is needed.

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Previous studies have clearly demonstrated the pathogenic role of food allergy, especially in children with atopic dermatitis (AD) (1). Egg, milk, peanut, soy, fish, and wheat are the most commonly offending foods (2). They are reported to account for 89% of the positive challenges in approximately 30% of children with AD (3, 4). A major problem, especially in wheat allergy, has been the lack of knowledge of the responsible allergens and therefore the difficulty in screening patients who would benefit from elimination diets. Studies with commercially available test mater-

ials for SPT and RAST have not always been satisfactory (5, 6).

Cereal allergy may develop after inhalation or ingestion. Inhaled cereal antigens among bakers result in baker's asthma (7). The main IgE response is directed to albumins and globulins, proteins soluble in neutral solutions (8), although IgE binding to water-insoluble wheat fractions has also been reported (9). Several studies have shown that the gliadin proteins are the most harmful antigens in celiac disease, resulting in an IgA- and IgG-antibody response after ingestion of

cereals (10–12). Another entity resulting from ingestion is cereal-dependent exercise-induced anaphylaxis. This is a severe reaction, in which the symptoms occur only after ingestion of food containing cereals followed by exercise. By an IgE-immunoblotting study, we found that wheat gliadin and the corresponding ethanol-soluble proteins of taxonomically closely related cereals were allergens that could cause this reaction (13).

Although cereals form an essential part of the daily diet and are basic foods that often cause allergic reactions or deterioration of AD in children, only a few studies have focused on the allergens of cereals (14, 15). In our recent study on cereal-allergic children with AD, we found, in addition to an intensive IgE reactivity to neutral/acidic soluble wheat peptides by immunoblotting, also a strong association between the positive wheat challenge and the positive skin prick test (SPT) with the ethanol-soluble gliadin (16).

In the present study, to investigate further the importance of gliadin peptides as allergens in AD, we analyzed sera from wheat-challenge-positive or negative-children and samples of adults with AD by IgE immunoblotting.

Material and methods

Patients

The sera were obtained from 18 children attending the Skin and Allergy Hospital of Helsinki University (Finland). They had been sent to the hospital because of severe AD and suspicion of food allergy. In addition to dermatitis, eight had asthma and two had allergic rhinitis. The seven adult patients were examined at the Skin and Allergy Hospital and the Department of Dermatology of Turku University. Six adults had severe, extensive, or intensively itching dermatitis, mainly of the head, neck, and shoulder type. The diagnosis of AD was established by the criteria of Hanifin & Rajka (17). One adult patient had urticaria and history of anaphylactic reactions. All had suspicion of cereal allergy. Seven of the children were girls and 11 boys; five of the adults were women and two men. The mean age of the children was 3 years (1–11 years) and of the adults 27 years (17–44 years). Each child underwent wheat challenge performed and followed up at the hospital. Before the challenges, the patients were kept on a cereal-free diet for at least 2 weeks. They were also treated with topical hydrocortisone if needed and emollients. The challenges were performed when the skin was clear from symptoms of AD.

Oral challenges

The wheat challenges were performed in an open way starting with 1 g of wheat porridge cooked in water, followed by 5 and 10 g. Two doses were given during the day, and the challenge was continued for 3

consecutive days up to 10 g twice daily. If no symptoms appeared, the children were freely fed wheat-containing foods at home, and later the diet was extended to include all the cereals. During the challenges, four children developed urticaria, one urticaria and wheezing, four erythema and pruritus, and four eczema. Five were negative in oral challenge. The adults followed a cereal-free diet for at least 1 month. The patient with exercise-induced urticaria/anaphylaxis became symptom-free; three patients with AD experienced relief and continued with the diet. In three adults, cereal exposure after the diet had no clear effect on the clinical picture.

The study protocol was approved by the ethics committees of the two hospitals.

Preparation of wheat antigens

NaCl suspension of wheat, purified gliadin for SPT, and the ethanol extract of wheat for immunoblotting studies were prepared as previously described in detail (13). The gliadin SPT solution was prepared as follows. Commercial gliadin (BDH, British Drug Houses, Poole, UK) was dissolved in a mixture of equal volumes of 96% ethanol and albumin diluent, and centrifuged at

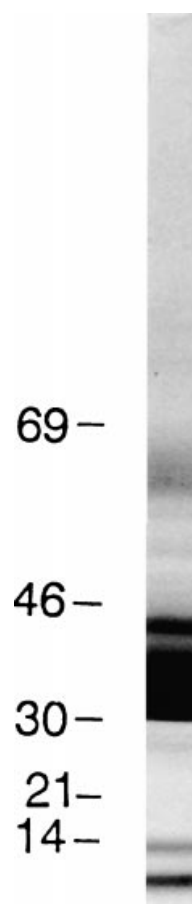


Figure 1. SDS-PAGE pattern of ethanol extract of wheat separated in 5–17% gradient gel and stained with Coomassie brilliant blue.

10 000 rpm for 30 min. A sample of the supernatant was then suspended with an equal amount of 85% glycerol and, in sterile conditions, passed through a 0.22- μ m Millipore filter to obtain the solution for SPT.

SPT

All patients underwent SPT with the NaCl suspension of wheat. Purified gliadin in ethanol was tested with the 1 mg/ml test solution (16). A wheal with a diameter of 3 mm and greater than half of that of the wheal caused by histamine hydrochloride (10 mg/ml) was regarded as positive, provided that the vehicle control appeared negative, and was graded as follows: ++ half of the size of, +++ equal to, or ++++ twice as large as the positive control.

Laboratory tests

Blood samples were taken simultaneously with the SPTs to be analyzed for specific IgE antibodies to wheat and gluten (CAP RAST, Pharmacia, Uppsala, Sweden) and serum total IgE (CAP RIA, Pharmacia, Uppsala, Sweden). A RAST score of >0.35 kU/l was regarded as positive.

SDS-PAGE and immunoblotting

The ethanol extract of wheat and molecular weight markers (Rainbow, Amersham, UK) were separated by SDS-PAGE, and the proteins were transferred to nitrocellulose sheets (Schleicher & Schnell, Dassel, Germany) in a Transphor apparatus (LKB, Bromma, Sweden), as previously described (13).

Results

Table 1 presents the characteristics of patients who underwent SPT and whose sera were used in RAST and total IgE determinations and in IgE-immunoblotting analysis against the ethanol extract of wheat (Fig. 2). The delay of reactions and clinical symptoms on wheat challenge in the children are illustrated in Table 1. The numbers of patients in Table 1 refer to corresponding numbers in Fig. 2.

SPT and RAST

Nos. 1–13 (Table 1) refer to challenge-positive children, who were positive to wheat, and 11/12 of whom were positive to gliadin in SPT. Nos. 14–18 were wheat-challenge-negative; 3/5 of them had a positive wheat SPT, but none displayed a positive gliadin SPT. Nos. 19–25 refer to adult patients, of whom four responded to a cereal-free diet (nos. 21, 22, 24, and 25). The last two (nos. 24 and 25) displayed a decrease in wheat/gluten RAST and total IgE values during follow-up. The figures of no. 24 for total IgE varied from 10 500 to 7150 kU/l, for wheat RAST from 41.9 to 16 kU/l, and for gluten RAST from 3.0 to 0.9 kU/l. For patient no. 25, the total IgE decreased from 5700 to 286 kU/l, and gluten RAST became negative from 2.8 kU/l.

SDS-PAGE and immunoblotting

Fig. 1 shows the SDS-pattern of the ethanol extract of wheat stained with Coomassie brilliant blue. Individual IgE immunoblots are shown in Fig. 2 against the ethanol wheat extract. A characteristic finding was that

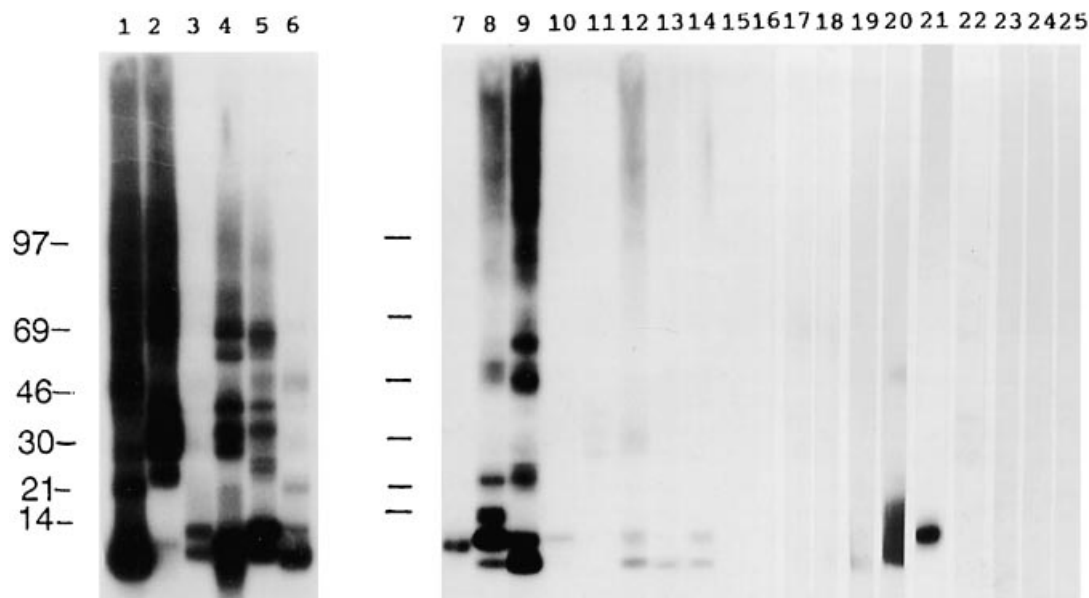


Figure 2. IgE immunoblots with patient sera against ethanol extract of wheat. Lanes 1–13) AD children with clinical wheat allergy; 14–18) non-wheat-allergic AD children; 19–25) adult AD patients, of whom nos. 21, 22, 24, and 25 responded to cereal-free diet. Patient numbers refer to corresponding numbers in Table 1.

Table 1.

Patient no.	Age (years)/sex	Symptom	Skin prick test		RAST kU/l		Total IgE kU/l	Challenge reaction	Delay of reaction
			Wheat	Gliadin	Wheat	Gluten			
1	3/F	AD/A	++++	++++	58.0	24.0	450	Urticaria	10 min
2	2/M	AD/A	++	+++	64.0	15.3	730	Urticaria	30 min
3	6/F	AD	+++	—	14.0	10.6	2000	Eczema	24 h
4	2/M	AD/A	++++	++++	44.0	11.8	990	Urticaria	15 min
5	1/F	AD	++++	+++	23.0	6.0	47	Erythema, pruritus	48 h
6	3/M	AD/A	++++	+++	13.0	8.2	110	Urticaria, wheezing	1 h
7	1/F	AD	++	++	13.0	2.9	210	Eczema	48 h
8	3/M	AD/A/R	++++	+++	32.0	13.6	1500	Erythema, pruritus	30 min
9	3/M	AD/A	+++	++	68.0	21.1	120	Urticaria	15 min
10	1/F	AD	+++	++	13.0	5.4	140	Eczema	72 h
11	2/M	AD	+++	+++	6.9	5.6	200	Erythema, pruritus	2 h
12	3/M	AD/A	++++	++++	52.0	4.0	2400	Eczema	72 h
13	11/M	AD/A/R	++	ND	14.0	10.4	140	Erythema, pruritus	3 h
14	4/F	AD	++	—	11.0	0.0	1500	Negative	—
15	2/M	AD	—	—	0.1	0.0	130	Negative	—
16	4/F	AD	++	—	11.0	0.0	1400	Negative	—
17	5/M	AD	++	—	3.0	0.0	280	Negative	—
18	3/M	AD	—	—	0.4	0.0	170	Negative	—
19	28/F	AD/U	+++	++	17.0	2.2	149		
20	30/F	AD/A	+++	++	30.0	27.8	700		
21	17/F	AD	+++	+++	36.0	20.0	11 300		
22	26/F	EIU/A	+++	+++	3.1	1.3	89		
23	25/F	AD/R	—	—	3.1	0.87	4420		
24	24/M	AD/R	—	—	41.9	3.0	10 500		
25	44/M	AD/R	+++	—	9.5	2.8	5700		

AD: atopic dermatitis; A: asthma; R: rhinitis; ND: not done; EIU/A: exercise-induced urticaria/anaphylaxis.

the IgE immunoblotting revealed small, below 14-kDa, heavily stained bands in sera from all challenge and gliadin-SPT-positive children with AD as well as in two gliadin-SPT-positive AD adults. The immunoblots were negative in seven out of eight patients with negative gliadin SPT. Heavily stained bands in the main gliadin area between 30 and 46 kDa were also notable, especially in the sera of challenge-positive children with positive gliadin SPT and high-gluten RAST. As a whole, the most intensive and broad staining appeared in sera with clearly elevated gluten RAST. The negative stainings were seen in sera with negative or only slightly elevated gluten RAST values. On the other hand, high total IgE values were not found to affect the immunoblots.

Discussion

Wheat proteins may be classified according to solubility into albumins, globulins, glutelins, and gliadins. Albumins and globulins are soluble in dilute neutral solutions, glutelins in dilute acids, and gliadins in aqueous ethanol. The glutelin and gliadin fractions together form wheat gluten.

According to the present study, gliadin seemed to be the significant allergen if wheat caused symptoms after ingestion, since children with IgE antibodies to gliadin measured by SPT or RAST responded to elimination.

In adults, the picture was more complicated and may reflect difficulties in following a strict diet and also development of tolerance. In addition, in adults, besides allergens, a number of other factors such as emotional stress, hormones, irritant substances, climatic factors, and microbial agents may be of importance for the course of AD.

The gliadin SPT results displayed good correlation with commercial gluten RAST, which seemed to contain also other wheat proteins besides gliadin, since it gave positive results also in gliadin-SPT-negative patients. This was evident in our earlier study on AD children, too (16). In the present study, a good correlation between the positive gliadin SPT and the positive IgE immunoblotting against gliadin was evident, and the most intensively stained blots were found in wheat-allergic children who also displayed a high RAST score for gluten (Table 1, Fig. 2). The correlation with food challenges was significant; 11/12 children with gliadin SPT and 13/13 with positive RAST were challenge positive, whereas all five SPT- and RAST-negative subjects were challenge-negative, although they displayed positive SPT and RAST results with other wheat proteins. In adults, the correlation showed a similar trend; however, only seven samples were analyzed.

It is evident that the antigens to detect IgE response to cereals have been of different quality. The starting material and processing of grain affects the protein

composition significantly. To optimize the amount of allergenic proteins, crushed whole-wheat grains in NaCl were used (18). The crude NaCl wheat suspension is a mixture of many proteins and seemed to serve as a good screening agent (13, 16), but wheat SPT or RAST alone was not associated with challenge positivity. The gliadin SPT solution was prepared from commercial gliadin and an ethanol extract of wheat was used in immunoblotting. The staining of the commercial gliadin preparation and the ethanol extract of wheat with IgE antibodies from the sera of gliadin-SPT-positive AD patients and patients with wheat-dependent exercise-induced urticaria/anaphylaxis revealed almost identical staining patterns (19).

As defined by staining with Coomassie brilliant blue, the ethanol extract contained the previously characterized major prolamin peptides (Fig. 1) (18). However, this extract contained also bands of less than 14 kDa, which were heavily stained by IgE antibodies from the sera of gliadin-SPT-positive AD patients (Fig. 2). These small peptides may represent previously uncharacterized gliadins, since no corresponding IgE immunoblotting against gliadin has been done with the sera of children with AD and cereal allergy. Interestingly, comparison of the present IgE immunoblots and IgA and IgG immunoblots of patients with celiac disease (20) against gliadin proteins revealed antibody binding mostly to peptides of the same mass. In celiac patients, both IgA and IgG antibodies bound also to this <14-kDa protein, although the most intensive staining among them occurred in the main gliadin area of 30–43 kDa. In the present study, a broader spectrum of gliadin proteins were stained by IgE with sera of AD children. These results suggest that the same antigenic epitopes of gliadin may cause either the IgE or the IgA/IgG immune response in different patient groups.

Gliadin peptides are known to damage the gut mucosa in celiac disease, resulting in an IgA and IgG class antigliadin antibody response (21, 22). Recently, we found by IgE immunoblotting that wheat gliadin and the

corresponding ethanol-soluble peptides in rye, barley, and oats were the allergens in patients with cereal-dependent exercise-induced anaphylaxis (13). In another Finnish study on cereal allergy in children, Räsänen et al. (23) also found that gliadin IgE antibodies can be frequently found with the RAST method and basophil histamine-release test, but no *in vivo* gliadin SPT or immunoblotting studies were performed in their study. Thus, these studies suggest that wheat gliadin is an important food allergen not only in patients with exercise-induced anaphylaxis but also in patients with AD and cereal allergy. Gliadin has so far been mostly linked to celiac disease, and its role as an IgE-mediated allergen has been overlooked. In AD, the portal of entry for the food allergens seems to be the gastrointestinal tract, where the proteins are exposed to various gastrointestinal conditions and enzymatic activities. It is well recognized that patients with baker's asthma do not usually react to cereal-containing food. Cross-reacting IgE antibodies between grass pollen and wheat proteins have also been reported (24). A study on wheat-dependent exercise-induced anaphylaxis showed by SPT that pepsin-digested proteins increased, and the trypsin digestion decreased the IgE reactivity of wheat allergens (25). Thus, the allergenic activity of gliadin could be enhanced in the stomach and maintained in the duodenum. A recent study on celiac disease showed that deamidation of gliadin by tissue transglutaminase promotes its binding to HLA-DQ2 on antigen-presenting cells and recognition by gut T cells (26). Thus, in susceptible patients with atopic background, the gliadin antigens could similarly act as immunomodulators, resulting in an IgE response.

In conclusion we showed that wheat-allergic AD patients have IgE antibodies against gliadin that can be detected by both SPT and the immunoblotting method, suggesting that gliadin peptides are important allergens and useful tools in screening patients with clinical allergy to ingested wheat. It is evident that further characterization and purification of these IgE-binding, ethanol-soluble peptides are needed.

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