

Food allergy to wheat: identification of immunoglobulin E and immunoglobulin G-binding proteins with sequential extracts and purified proteins from wheat flour

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Summary

Background Cereal-associated allergy is particularly considered a serious problem, because cereals are essential in our daily diet. Wheat proteins are classified into albumins, globulins and prolamins (insoluble gliadins and glutenins).

Objectives Our objectives were to study the involvement in food allergy to wheat of these different protein types by using purified fractions and to identify those binding IgE and IgG antibodies.

Methods Sera were obtained from 28 patients with food allergy to wheat. Albumins/globulins, gliadins and glutenins were obtained by sequential extraction based on differential solubility; α -, β -, γ - and ω -gliadins and low molecular weight (LMW) and high molecular weight (HMW) glutenin subunits were purified by chromatography. IgE binding to these extracts and fractions were analysed by radioallergosorbent test (RAST), and immunoblotting; IgG binding was detected by enzyme-linked immunosorbent assay (ELISA).

Results In RAST, 60% of sera were shown to have specific IgE antibodies against α -, β -gliadins and LMW glutenin subunits, 55% to γ -gliadins, 48% to ω -gliadins and 26% to HMW glutenins. Immunoblotting analysis confirmed results obtained in RAST concerning LMW and HMW glutenin subunits and showed that 67% of patients have IgE antibodies to the albumin/globulin fraction.

Conclusion Results obtained in the different tests showed common features and in agreement with other studies indicated the presence of numerous allergens in food allergy to wheat; α -, β -, γ - and ω -gliadins, LMW glutenin subunits and some water/salt-soluble proteins appeared as major IgE binding allergens, whereas HMW glutenins were only minor allergens. The same type of antigenic profile against gliadins and glutenins was observed with IgG antibodies. Important sequence or structural homologies between the various gliadins and LMW glutenin subunits could certainly explain similarity of IgE binding to these proteins.

Keywords food allergy, gliadins, glutenins, IgE, IgG, wheat

Submitted 9 April 2002; revised 1 October 2002; accepted 30 October 2002

Introduction

The number of patients suffering from food allergy is increasing throughout the world, and corresponds to 1–5% of the population [1]. Because of the extensive use of wheat and related cereals in our daily diet, cereal-associated allergies are particularly considered as a serious problem. Hypersensitivity reactions to wheat flour occur both by inhalation (baker's asthma) and ingestion (food allergy and coeliac disease) but may also develop in a few cases by contact [2, 3].

Wheat kernel contains numerous proteins, several of which are involved in the different pathologies. On the basis of differential solubility, wheat proteins can be classified into water/

salt-soluble albumins and globulins and water/salt-insoluble gliadins and glutenins [4]. Albumins and globulins from wheat are not very well characterized for the moment with the exception of α -amylase and trypsin inhibitors [5] and of lipid binding proteins [6]. Singh et al. [7] have identified by N-terminal sequencing in the water-soluble extract (albumins) of Chinese Spring flour a number of bands between 13 and 18 kDa corresponding to α -amylase inhibitors and bands at 37 and 62 kDa corresponding, respectively, to a peroxidase precursor and a serine carboxypeptidase; in the salt-soluble extract (globulins), many bands with various molecular weights (MW, 20, 37, 38 and 54 kDa) were homologous to barley globulins. Composition and structure of gliadins and glutenins, known as prolamins, have been better characterized [8, 9]. Gliadins are monomeric proteins and account for about 30% of wheat grain proteins; they are classified into α -, β -, γ - and ω -gliadins according to their sequences and electrophoretic mobility in acid–polyacrylamide gel electrophoresis (PAGE); the MW of α -, β - and γ -gliadins range from 30 to 40 kDa; that of ω -gliadins

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range from 40 to 50 kDa [10] although their apparent MW in sodium dodecyl sulphate (SDS)-PAGE is much higher (55–75 kDa). Slow- and fast-moving ω -gliadin components can be observed in acid-PAGE and were designated by Kasarda et al. [11] ω 1, ω 2 (slow-moving) and ω 5 (fast-moving) in relation with their mobility and their N-terminal sequences. Glutenins [9] account for about 35% of total proteins and correspond to polymeric proteins linked by intermolecular disulphide bridges; they are composed of high molecular weight (HMW) subunits (apparent MW in SDS-PAGE range from 80 to 120 kDa) and of low molecular weight (LMW) subunits; LMW-GS are further divided into three groups of subunits: the B-group (subunits of 42–51 kDa), the C-group (30–40 kDa), including subunits related to $\alpha\beta$ - and γ -gliadins and the D-group (60–75 kDa) containing ω -gliadin-like subunits. The main features of prolamins are their wide polymorphism, their similar organization into repetitive and non-repetitive domains and the presence of extensive sequence homologies between the different gliadins and glutenins.

Both water- and salt-soluble proteins and prolamins were found to be involved in food allergy to wheat [12–15]. Watanabe et al. [16] isolated from a chymotryptic hydrolysate of gluten an allergenic peptide [(SQQQ(Q)PPF) $\times$ 4] highly homologous to the repetitive domain of a LMW glutenin subunit. Omega 5-gliadin, α - and γ -gliadins or homologous prolamins in rye and barley were recognized by IgE from adults with wheat-dependant exercise-induced anaphylaxis (WDEIA) [17–19] or children with immediate allergy to wheat [20]. Maruyama et al. [21] also observed IgE binding to gliadin, glutenin and salt-soluble fractions of wheat and a particular high reactivity of IgE antibodies with recombinant α - and γ -gliadins and LMW glutenin subunits. Sudhalekshmy et al. [22] identified a major allergen of 65 kDa in phosphate-buffered saline (PBS)-soluble wheat proteins, and Kusaba-Nakayama et al. [23] and James et al. [24] observed IgE binding to α -amylase inhibitors around 14–15 kDa.

Several of the previous studies on food allergy to wheat [12–15, 25] were carried out using water/salt-soluble/insoluble fractions from wheat flour in which IgE binding components were not precisely characterized; in other cases a special emphasis was made on a few fractions detected by patient IgE [16, 18, 20, 23, 24] or on recombinant antigens that could be not fully representative of native proteins [21]. The aim of our work was to extend previous studies by identifying IgE binding proteins for a large collection of sera from patients with accurate diagnostic of food allergy to wheat and by studying the involvement of several fractions representative of the different wheat protein classes. Thus, we examined antibodies in sera from children as well as from adults presenting a large spectrum of clinical symptoms as atopic dermatitis, asthma, urticaria or anaphylaxis. To study the involvement of all protein types present in wheat flour, we used sequential fractions of wheat proteins obtained by differential solubility but also highly purified fractions representative of all prolamins classes.

The presence of IgG antibodies to wheat proteins in patients allergic to wheat had been rarely examined although these antibodies may reflect an intrinsic antigenicity for some major allergens [26]. We have thus included in our study the characterization of IgG reactivity against the prolamins fractions.

Materials and methods

Human sera

Sera were obtained from 28 patients (14 children and 14 adults) with food allergy to wheat, suffering from various symptoms (atopic dermatitis, asthma, urticaria, eczema, digestive symptoms and one case of anaphylaxis). Food allergy to wheat was established by evidence of IgE-dependent sensitization [prick tests and radioallergosorbent test (RAST) cap system] and by standardized oral challenge (double- or single-blind, placebo-controlled, food challenges) [D. A. Moneret-Vautrin et al., to be published]. Twenty-three sera from patients receiving a transplant or allergic to antibiotics and without food allergy were used as controls.

Purification of gliadin and glutenin fractions

Crude gliadins were extracted from flour Etoile de Choisy with 70% (v/v) ethanol. α -, β -, γ - and fast- and slow-moving ω -gliadin fractions were then purified by ion-exchange chromatography and reversed-phase high-performance liquid chromatography (HPLC) as described by Popineau et al. [27, 28]. HMW and LMW glutenin subunits were prepared from defatted flour Soisson according to the procedure of Verbruggen et al. [29]. Briefly, a pre-extraction of gliadins was performed with 50% (v/v) propanol-1 at room temperature. Glutenin subunits were solubilized from the residue at 60 °C with 50% (v/v) propanol containing 1% (w/v) dithiothreitol (DTT). The HMW glutenin subunits were precipitated selectively by increasing the propanol concentration to 60%, and the LMW glutenin subunits then precipitated with a final concentration of 85%. The composition and purity of these fractions were controlled by SDS-PAGE as described previously [30]; gliadin fractions were also analysed by acid-PAGE [31, 32].

Sequential extraction of wheat proteins

Wheat proteins were extracted from 100 mg of flour (cultivar Etoile de Choisy) according to the sequential procedure of Osborne [4], adapted by Nicolas et al. [33]. Albumins/globulins were extracted in 3 mL 0.05 M phosphate/0.1 M NaCl buffer pH 7.8 during 1 h at 4 °C with constant stirring. After centrifugation (20 000 g for 20 min at 4 °C), the supernatant was collected and frozen. Gliadins were extracted in 3 mL of 70% (v/v) ethanol at room temperature for 1 h. The sample was centrifuged (20 000 g for 20 min at 4 °C) and the supernatant was dried and frozen. Wheat glutenins were extracted as described previously by Singh et al. [34]. The pellet was washed with 70% (v/v) ethanol and was resuspended in 1350 μ L 0.1 M Tris-HCl buffer pH 8.5 containing 50% propanol-2 and 1% (w/v) DTT (1 M). After 30 min at 60 °C, 450 μ L of the same solution containing 1.4% 4-vinylpyridine (v/v) were added. After 1 h at 60 °C and overnight at room temperature, the sample was centrifuged (20 000 g for 20 min at 4 °C) and the supernatant was collected and frozen.

Immunoblotting

Proteins from albumin/globulin and glutenin fractions were separated by SDS-PAGE in reducing conditions on 15% and 10% acrylamide gels, respectively [30]. Albumins/globulins were concentrated and solubilized in Tris-HCl (63 mM) buffer pH 6.8 ($\times$ 5) containing 2% (w/v) SDS, 10% (v/v) glycerol and

bromophenol blue. Proteins were reduced by 5% (v/v) 2-mercapto-ethanol and 200 μ L were loaded on the gel in a unique well. Glutenin fractions were diluted in Tris-HCl buffer ($\times 2$) and 90 μ L were loaded. After separation, proteins were transferred to nitrocellulose sheets (0.2 μ m, Sartorius, Göttingen, Germany) in Tris (25 mM)/SDS (0.1%, p/v)/glycine (192 mM)/ethanol (20%, v/v) buffer at 250 mA during 2 h for albumins/globulins and 2.5 h for glutenin fractions. Before immunoblotting, proteins from gliadin extract were separated by acid-PAGE in which α -, β -, γ - and ω -gliadin components exhibit more different mobilities than in SDS-PAGE. They were dissolved in 300 μ L 0.025 M acetic acid buffer containing 20% (v/v) glycerol and 6 M urea; 60 μ L of gliadins were then loaded on acid-PAGE gels [31, 32] and migration was performed towards the cathode. After incubation of the gel for 20 min in the transfer buffer, the proteins were transferred for 1 h to nitrocellulose sheets in the same buffer as for the other fractions. After transfer, nitrocellulose sheets were cut and strips were blocked for 4 h at room temperature in 2% polyvinylpyrrolidone (PVP-40, Sigma P-0930, Sigma, St Louis, MO, USA) in Tris-buffered saline (TBS)-Tween 0.1%. After three washes with TBS-Tween-20 0.1%, nitrocellulose strips were incubated overnight with patient and control sera diluted 1 : 10 in blocking buffer. Strips were then washed three times with washing buffer and incubated overnight at room temperature with ^{125} I-labelled anti-human IgE (Immunotech, Luminy, France) or for 4 h with alkaline phosphatase-labelled goat anti-human IgE (Sigma). IgE binding was revealed by a 10-day autoradiography at -25°C with R-Biomax MS Kodak (Rochester, NY, USA) film combined with an intensifier screen Biomax. Alkaline phosphatase activity was revealed by incubation of the strips in Tris 0.1 M, NaCl 0.1 M, MgCl_2 0.05 M buffer pH 9.5 containing 0.33 mg/mL nitroblue-tetrazolium and 0.165 mg/mL 5-bromo-4-chloro-3 indolyl phosphate at room temperature for 15 min. This reaction was stopped in water.

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) was performed from stock solutions of purified prolamins. α -, β -, γ - and ω -gliadins fractions were dissolved at 1 mg/mL in 70% (v/v) ethanol; glutenin subunits were solubilized at the same concentration in 0.05 M carbonate buffer pH 9.6 containing 0.1% (w/v) SDS and 0.2% (v/v) 2-mercapto-ethanol. Purified prolamins fractions were coated overnight at 37°C to the wells of microtitre plaques (100 μ L/well, Falcon, Franklin Lakes, NJ, USA) at a concentration of 1 μ g/mL for prolamins. After each step of the test three washes were performed with phosphate-buffered saline (PBS) buffer containing 0.05% (v/v) Tween-20. The wells were saturated with 0.5% gelatine (Sigma, G-2500) in PBS-Tween for 1 h at 37°C and then incubated for 2 h at 37°C with patient and control sera 1 : 500 in PBS/Tween/gelatine. Alkaline phosphatase-labelled goat anti-human IgG (γ -chain specific, Sigma A-5403) diluted 1 : 1000 were added for 2 h at 37°C . The alkaline phosphatase reactivity was detected by adding the substrate (p-nitrophenylphosphate, Sigma N-2765) in 1 M Tris-HCl pH 9.8 buffer. The absorbance was read at 405 nm with an automated ELISA reader after 30 min reaction. Sera were considered to be positive for IgG binding when the absorbance values were two times higher than the absorbance values of wells containing no antigen.

Radioallergosorbent test

IgE antibodies to gliadins and glutenins were measured by direct RAST. The gliadins and glutenins from stock solutions were coupled to CNBr-activated paper discs at 0.260 ng/mL [35]. Sera diluted 1/10 were incubated for 3 h at room temperature (20°C) and specific IgE were further detected by ^{125} I anti-human IgE (Immunotech, Luminy, France). The assay was calibrated using monoclonal anti-human IgE discs and a control curve with serial dilutions of purified human myeloma IgE ranging from 100 to 0.5 UI/mL. Results were expressed in UI/mL.

Results

Purity of individual prolamins fractions and composition of sequential extracts

The composition and purity of gliadin and glutenin fractions used in ELISA and RAST were controlled by SDS-PAGE (Fig. 1a). HMW glutenin fraction purified from cultivar Soisson contained five subunits migrating between 75 and 110 kDa (lane 1) named 1Ax2*, 1Dx5, 1Bx7, 1By8 and 1Dy10 in order of increasing mobility [36]; it was not contaminated by gliadins or by LMW glutenin subunits. The LMW glutenin fraction (lane 2) contained mainly B type subunits migrating between 40 and 45 kDa and lower concentration of C type subunits between 30 and 40 kDa; no traces of HMW glutenin subunits could be detected in this fraction or in the gliadin preparations (lanes 3–6). Acid-PAGE analysis of α -, β -, γ - and ω -gliadin fractions (Fig. 1b) showed they only contained a single gliadin type (except the β -gliadin preparation containing traces of γ -gliadins).

The composition of sequential extracts were analysed after electrophoresis and Coomassie blue staining. In the gliadin extract several bands could be seen after acid-PAGE corresponding to α -, β -, γ - and ω -gliadin in order of increasing mobility (Fig. 2c). In the glutenin extract, four HMW subunits and B- and C-type LMW subunits were visible after staining (Fig. 2b). Numerous components between 10 and 94 kDa could be observed in the albumin/globulin extract, with major bands visible around 15 kDa and between 25 and 67 kDa (Fig. 2a). Anti-peptide antibodies specific for α -, β -, γ - and ω -gliadins and for LMW and HMW subunits [30, 37] were used in immunoblotting to analyse the presence of contaminating gliadins and glutenin subunits in the NaCl extract; they detected significant amounts of gliadins and LMW subunits (not shown).

Reactivity of IgE type antibodies from patients with food allergy to wheat

Reactivity of IgE antibodies for gliadin, glutenin and albumin/globulin fractions was examined by means of two complementary techniques: RAST and immunoblotting. Antigen immobilized in RAST were the α -, β -, γ - and total ω -gliadin and LMW and HMW glutenin fractions purified by chromatography. Immunoblotting analyses were performed with the albumin/globulin, gliadin and glutenin fractions obtained by sequential extraction. For glutenins it permitted us to see eventual differences in subunit recognition and for gliadins, it allowed us to complete the results obtained in RAST on very selective gliadin components by analysing an extract containing several α -, β -, γ - and ω -gliadin components from the flour.

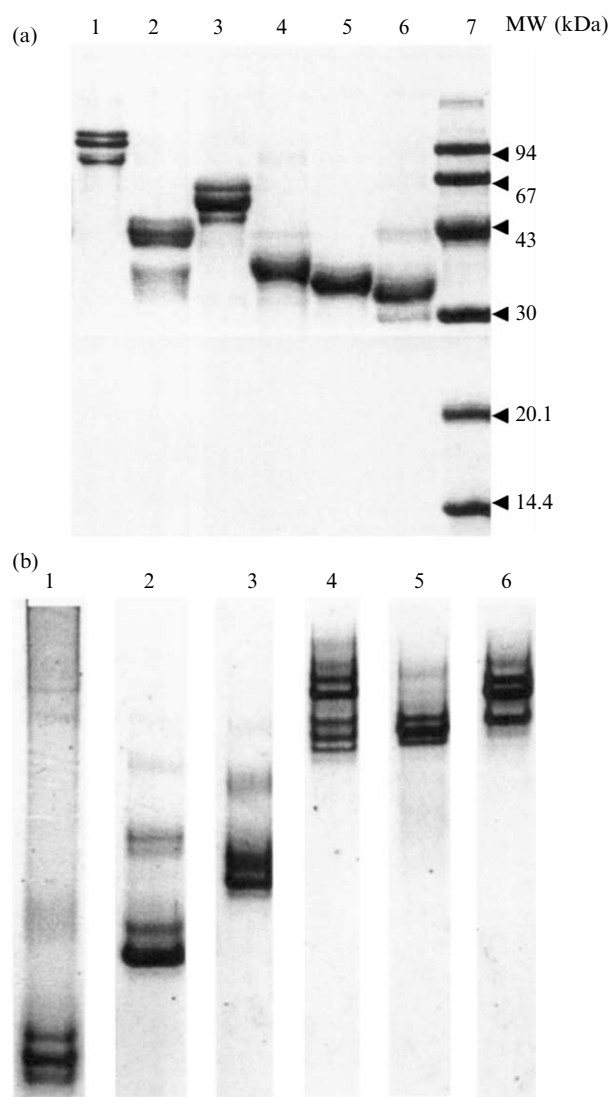


Fig. 1. Analysis by electrophoresis of the composition of purified gliadin and glutenin fractions. (a) Separation of the different fractions by SDS-PAGE: lane 1, HMW glutenin subunits; lane 2, LMW glutenin subunits; lane 3, total ω -gliadins; lane 4, γ -gliadins; lane 5, β -gliadins; lane 6, α -gliadins; lane 7, Molecular weight markers. (b) Separation of gliadin fractions by acid-PAGE: lane 1, α -gliadins; lane 2, β -gliadins; lane 3, γ -gliadins; lane 4, total ω -gliadins; lane 5, fast ω -gliadins; lane 6, slow ω -gliadins.

Control sera were included in all these analyses, but none of them had IgE antibodies directed against the various wheat protein fractions analysed.

Reactivity of IgE antibodies with gliadin fractions

The presence of IgE antibodies against gliadin fractions was analysed by RAST and immunoblotting after acid-PAGE. The results obtained in RAST showed that 67% of patients among the 27 analysed have IgE antibodies to gliadin fractions, 60% to α - and β -gliadins, 55% to γ -gliadins and 48% to total ω -gliadins. Most sera positive to gliadins could be classified into classes 3 and 4, and a few of them into classes 5 and 6 (Table 1). In immunoblotting (Table 2, Fig. 2c), only 7% of the 27 sera tested showed binding to α - and γ -gliadin components and 20% to β - and ω -gliadins. The ω -gliadin components detected by the

sera were of fast-moving type. The slow-moving components were not recognized (Fig. 2c).

Reactivity of IgE antibodies with glutenin fractions

The presence of IgE antibodies against glutenin fractions was analysed by RAST and immunoblotting after SDS-PAGE. Table 1 showed that 60% of sera on the 27 analysed contained IgE antibodies against LMW glutenin subunits and only 26% against HMW glutenin subunits. Most responses belonged to classes 3, 4 and 5 for LMW glutenin subunits, and to class 3 for HMW subunits. In immunoblotting (Table 2, Fig. 2b), 52% of patients in 27 analysed showed IgE antibodies to LMW glutenin subunits and 15% to HMW glutenin subunits. All sera positive to the glutenin extract detected the B-type LMW subunits, with high intensity for most of them, whereas C-type subunits were not always recognized. Staining of HMW subunits was always weak. Some sera ($n = 4$) also detected components migrating around 60 kDa corresponding to ω -gliadins present in the glutenin fraction.

Reactivity of IgE antibodies with albumin/globulin fraction

The presence of IgE antibodies against proteins of the albumin/globulin extract was analysed by immunoblotting after SDS-PAGE (Table 2). The results indicated that 67% of the 27 sera tested were positive against this fraction. Figure 2(a) showed an example of these results; for most positive sera, responses were observed against components between 30 and 70 kDa with intense staining for some sera around 32, 38, 43, 48 and 70 kDa; three sera recognized a major component at 15 kDa and one serum reacted with numerous bands between 12 and 70 kDa.

Reactivity of IgG type antibodies from patients with food allergy to wheat

The reactivity of IgG type antibodies against purified prolamin fractions was characterized by ELISA (Fig. 3). Out of the 25 sera analysed, 64% contained IgG antibodies to these fractions, 64% to α - and β -gliadins, 36% to γ -gliadins, 40% to LMW glutenin subunits, 32% to ω -gliadins and 16% to HMW glutenin subunits (Table 2). Nineteen control sera were also analysed in ELISA; IgG antibodies binding α - and β -gliadin fractions could be detected in 16% of them and IgG binding LMW glutenins in 11%.

Discussion

We analysed the antigen repertoires of IgE- and IgG-type antibodies from 28 patients with food allergy to wheat flour. Antibody reactivity was characterized in RAST and immunoblotting by using albumin/globulin, gliadin and glutenin fractions obtained from sequential extraction of wheat flour as well as individual gliadin and glutenin components purified by chromatography. In RAST, 48–60% of sera were shown to have specific IgE antibodies against α -, β -, γ - or ω -gliadins as well as LMW glutenin subunits, whereas only 26% of them detected HMW subunits. The intensity of responses also differed between these two classes of prolamins; positive results in RAST against gliadins and LMW glutenin subunits were of classes 3–6, whereas those against HMW subunits were of classes 2–3.

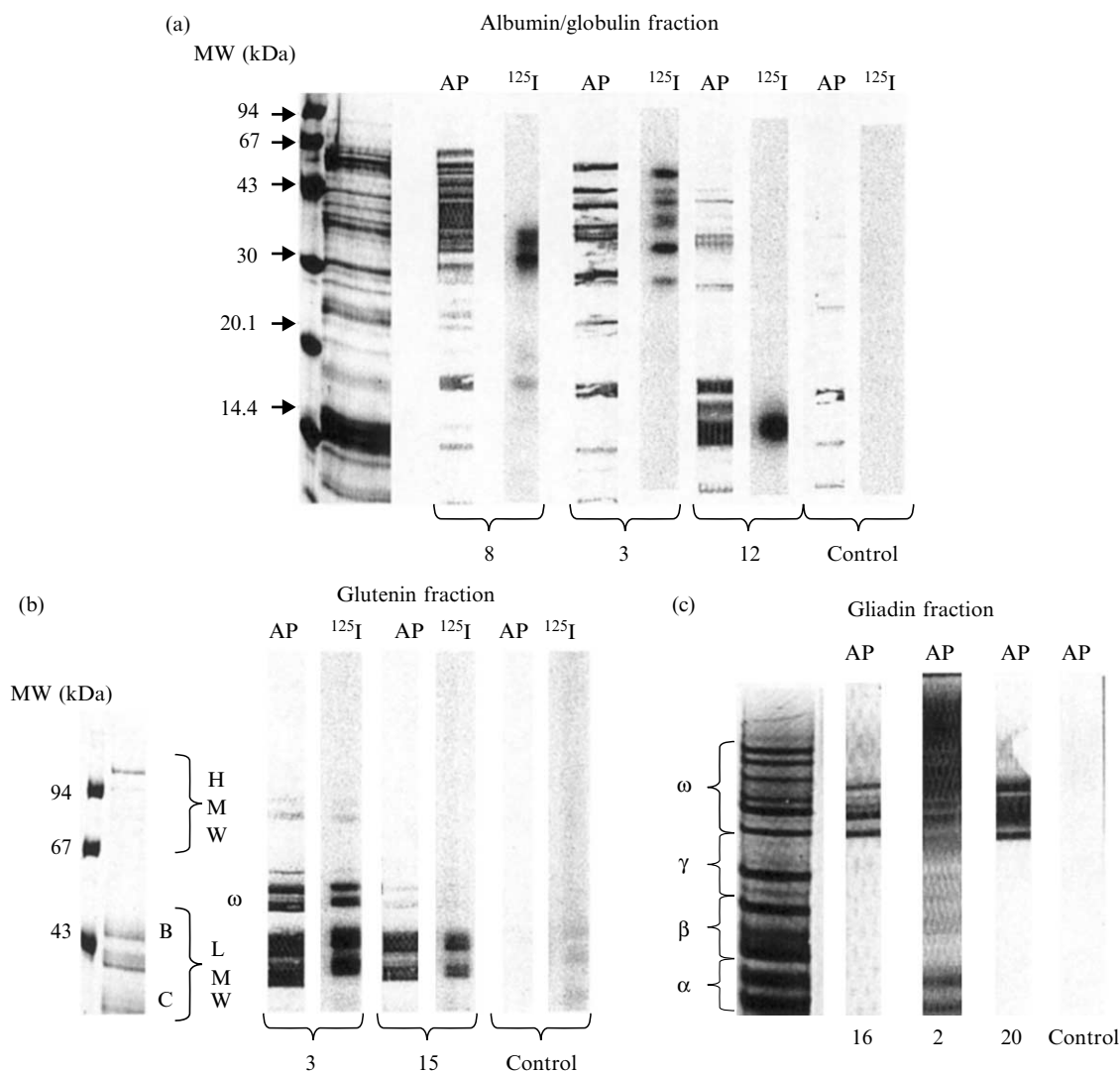


Fig. 2. Examples on a few sera of IgE reactivity in immunoblotting against (a) albumin/globulin, (b) glutenin extracts separated by SDS-PAGE and (c) gliadins separated by acid-PAGE. IgE binding was revealed by anti-human IgE antibodies labelled either with alkaline phosphatase (AP) or with ^{125}I .

Immunoblotting analysis confirmed results obtained in RAST concerning the glutenin subunits but not those concerning gliadins. The discrepancy between immunoblotting and RAST results for the gliadin fraction was certainly due to the use of a different electrophoretic system (in acidic buffer) from the one used for glutenin subunit separation (SDS-PAGE), resulting in a low adsorption of these proteins on nitrocellulose sheets and thus in a decrease in sensitivity; recognition of gliadin by IgE antibodies was thus underestimated by immunoblotting. Results obtained in immunoblotting with the albumin/globulin extract showed that 67% of wheat allergic patients have IgE antibodies directed against proteins (mainly in the migration zone between 30 and 70 kDa) present in this extract. For about 30% of sera we could not detect IgE binding in the RAST and immunoblotting analysis performed.

Our results were in good agreement with those from other studies on food allergy to wheat regarding IgE reactivity with gliadins and LMW glutenin subunits [15–21, 25]. We also observed IgE binding to HMW glutenin subunits although it was only rarely reported. Analysis by immunoblotting of IgE

binding to the albumin/globulin extract showed here that several components were recognized in this fraction, particularly at 15, 32, 38, 43, 48 and 70 kDa. The 15 kDa protein could correspond to wheat α -amylase inhibitor as IgE binding to this protein was mentioned in patients with allergy after ingestion of wheat [23, 24]. Singh et al. [7] identified sequences of ω - and α/β -gliadins among water-soluble proteins at 68 and 39 kDa, respectively. In our study, four sera reacted strongly in immunoblotting with ω -gliadins present in gliadin and glutenin extracts; in the albumin/globulin fraction these sera also bind to a 70-kDa band that might correspond to an ω -gliadin component. In this fraction, some IgE binding components between 30 and 45 kDa could correspond to contaminating α -, β - and γ -gliadins or LMW glutenin subunits as such proteins were detected in the extract by polyclonal antibodies specific for these proteins.

These results (Table 2) allowed us, in accordance with the other studies, to propose an antigenic profile for IgE antibodies in food allergy to wheat composed of several major allergens: α -, β -, γ -, ω -gliadins, LMW glutenin subunits and some proteins

Table 1. RAST analysis performed on 27 sera of wheat-allergic patients and two control sera

Sera	Levels of anti IgE ¹²⁵ I radioisotope uptake (UI/mL)					
	α -gliadin	β -gliadin	γ -gliadin	ω -gliadin	HMW glutenin	LMW glutenin
1	82/cl 5	79.5/cl 5	59.5/cl 5	32/cl 4	8/cl 3	40/cl 4
2	ND	ND	ND	ND	ND	ND
3	58/cl 5	45/cl 4	28/cl 4	124/cl 6	3.6/cl 3	65/cl 5
4	0	0	4.9/cl 3	5.1/cl 3	0	4.4/cl 3
5	0	0	0	0	0	0
6	0	0	0	0	0	0
7	0	0	0	0	0	0
8	189/cl 6	205/cl 6	103/cl 6	28/cl 4	22.5/cl 4	103/cl 6
9	33/cl 4	23/cl 4	21/cl 4	8/cl 3	0	15/cl 3
10	0	0	0	0	0	0
11	0	0	0	0	0	0
12	76/cl 5	3.7/cl 3	0	6.2/cl 3	0	3.2/cl 2
13	0	0	0	0	0	0
14	12/cl 3	9/cl 3	8/cl 3	3.8/cl 3	3.4/cl 2	8.5/cl 3
15	5.6/cl 3	0	0	0	0	3.9/cl 3
16	10/cl 3	0	32/cl 4	52/cl 5	0	15/cl 3
17	0	9.2/cl 3	0	0	0	0
18	12/cl 3	10.5/cl 3	9/cl 3	0	0	10/cl 3
19	36/cl 4	35/cl 4	49/cl 4	14/cl 3	0	15/cl 3
20	48/cl 4	28/cl 4	31/cl 4	76/cl 5	7.5/cl 3	59/cl 5
21	29/cl 4	19/cl 4	23/cl 4	15/cl 3	0	13/cl 3
22	12/cl 3	8.5/cl 3	4/cl 3	3.5/cl 3	0	12/cl 3
23	0	0	0	0	0	0
24	40/cl 4	16/cl 3	34/cl 4	62/cl 5	0	39/cl 4
25	12/cl 3	15/cl 3	7/cl 3	0	0	24/cl 4
26	0	0	0	0	0	0
27	14/cl 3	14/cl 3	5.1/cl 3	6.1/cl 3	4.2/cl 3	14/cl 3
28	0	0	0	0	0	0
Cont 1	0	0	0	0	0	0
Cont 2	0	0	0	0	0	0

ND, not determined; cl, class.

Table 2. Comparison of percentages of sera giving positive results for IgE or IgG binding to different wheat proteins by ELISA, RAST or immunoblotting

Techniques used		α -gliadin	β -gliadin	γ -gliadin	ω -gliadin		HMW glutenin	LMW glutenin	Alb/Glob
					Fast ω -gliadin	Slow ω -gliadin			
IgE									
<i>n</i> = 27	RAST	60%	60%	55%		48%	26%	60%	ND
<i>n</i> = 27	Immunoblotting	7%	20%	7%	20%	0%	15%	52%	67%
IgG									
<i>n</i> = 25	ELISA	64%		36%		32%	16%	40%	ND

ND, not determined.

of the water/salt extract (that comprise albumins, globulins and some co-extracted prolamins) and of minor allergens: HMW glutenin subunits. These allergens appeared to be specific for IgE antibodies of patients with food allergy to wheat as control sera did not contain IgE antibodies against the wheat proteins analysed. No clear relationship between IgE antigenic profile and clinical manifestations could be found in this patient cohort as well as no differences between children and adults. Palosuo et al. [18, 20] underlined a special involvement of ω 5-gliadin in adults with WDEIA and children with immediate hypersensitivity to ingested wheat. We demonstrated here reactivity to the

ω -gliadin fraction in other clinical contexts as urticaria in adults or atopic dermatitis in children.

Among the 18 sera positive in RAST against prolamins, 30% reacted with all the prolamins classes, 60% with all classes except HMW glutenin subunits and 80 and 89% detected at least four or three antigens, respectively, among α -, β -, γ -, ω -gliadins and LMW glutenin subunits. Very few sera reacted only to a single prolamins class. It is thus interesting to note that a wide range exist in patient IgE reactivity probably linked to important sequence homologies between prolamins. Many sequence homologies exist between gliadin and glutenin repetitive domain

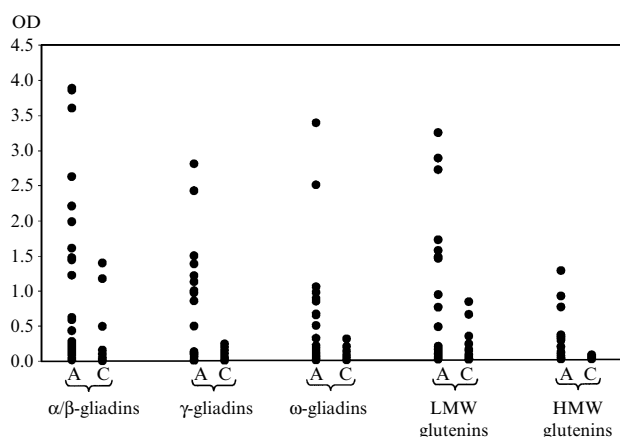


Fig. 3. ELISA performed on 25 sera from wheat-allergic patients (A) and 19 control sera (C) to analyse IgG reactivity against purified prolamin fractions adsorbed on microtitre plates. Each point represent the average of values obtained in two separated assays.

which are based upon repetitions of motives rich in Gln, Pro and Phe: PQQPY + PQQPFP ($\alpha\beta$ -gliadins), PFPQ₁₋₂(PQQ)₁₋₂ (γ -gliadins), PFPQ₁₋₂PQQ (ω 2-gliadins), PQQPPFS + QQQPVL (LMW glutenin subunits), PGQGQQ + GYYPTSP/LQQ (HMW subunits). Tanabe et al. [38] showed that a major epitope for IgE binding to gluten hydrolysate was the pentapeptide QQQPP belonging to the repetitive domain of LMW glutenin subunits. Cross-reactions at the level of the repetitive domains could explain why the various gliadins and the LMW glutenin subunits are recognized to the same extent and with quite similar intensities and why it is not the case of HMW glutenin subunits. Previous studies have shown that antibodies directed to repetitive motives of $\alpha\beta$ and γ -gliadins recognized almost equally well all the gliadin classes and cross-reacted with LMW glutenin subunits but did not detect HMW glutenins [39]. Sequence alignment also revealed the presence of three conserved regions called A, B and C in the structure of non-repetitive domains of prolamins and in that of α -amylase and trypsin inhibitors and of some 2S albumins [40,41]; these regions may be also responsible for cross-reactions between prolamins, especially sequences in the environment of very conserved cysteines. The presence of common epitopes in gliadins may also be explained by strong homologies in the hydrophobicity and in the conformation of non-repetitive domains of $\alpha\beta$ and γ -gliadins which appeared to be folded and stabilized by three or four intramolecular disulphide bonds [42]. Sequence homologies between LMW glutenin subunits and $\alpha\beta$ and γ -gliadins and secondary structure studies point to the same type of conformation for these proteins [43]. On the contrary, three-dimensional models of HMW glutenin subunits indicated quite different conformations for these molecules which possess a much longer repetitive domain than the other prolamins as well as a different repartition of non-repetitive sequences. Finally, the higher allergenicity of gliadins and LMW subunits compared to HMW subunits may also be due to the higher proportion of these prolamins in flour (gliadins: 30–40% of total protein; LMW subunits: 20–30%; HMW subunits: about 10%).

In addition to common epitopes, it is probable that IgE antibodies may also be elicited by epitopes specific of prolamin classes or of individual prolamin components. It is interesting

to note that α - and β -gliadins are considered to have identical sequences and to be encoded by the same loci but differences in reactivity may be observed between these proteins; indeed all sera that reacted with the α -gliadin fraction also reacted with the β -gliadin fraction, with the exception of two sera positive with the α component but not with the β one; on the contrary, one serum detected the β -gliadin fraction but none of the other prolamin fractions.

We also analysed the reactivity of IgG antibodies from 25 patients and observed that the different classes of gliadins and LMW glutenin subunits were also major antigens for IgG antibodies, whereas HMW subunits were minor antigens. This indicated that in food allergy to wheat, antigenic profiles of IgE and IgG antibodies directed against prolamins were quite similar. In the case of IgG, responses against prolamins could also be encountered for some control sera; however, the percentage of sera containing IgG to prolamin fractions and the intensity of responses were far smaller for controls than for allergic patients. It is interesting to note that four of the sera showing no response in RAST to prolamin fractions contained IgG antibodies to these antigens. As in our case, Szabo and Eigenmann [24] found similar fraction-specific IgE and IgG antibody-binding profiles to the major cow's milk or peanut antigens as well as the presence of IgG antibodies in non-allergic individuals. A similar pattern of reactivity was also observed for IgG antibodies from pre-ruminant calves fed on hydrolysed wheat proteins (JP Lalles et al., manuscript in preparation) which displayed high responses to albumin/globulin, gliadin and LMW glutenin subunit fractions and only few weak responses to HMW subunits. Similarity of IgG responses against prolamins in wheat-allergic patients and pre-ruminant calves is a confirmation that the intrinsic antigenicity of gliadins and LMW glutenin subunits is higher than that of HMW glutenin subunits.

Conclusion

According to previous studies, these data confirm the presence of numerous classes of allergens in food allergy to wheat: major allergens were found among gliadins and LMW glutenin subunits as well as among water/salt-soluble proteins and minor allergens were found in the HMW glutenin subunits. Similar antigenic profile for prolamins was observed with IgG antibodies. These results pointed out the wide range of IgE response to wheat prolamins as 80% of the sera analysed in this study reacted with at least four antigens simultaneously among α -, β -, γ -, ω -gliadins and LMW glutenin subunits; this polymorphism is probably linked to numerous sequence homologies that exist between prolamins and particularly between the different classes of gliadins and the LMW glutenin subunits. Contrarily to coeliac disease, where immunological events seemed to be mainly elicited by gliadin and glutenin repetitive peptides, food allergy to wheat would certainly involved non-repetitive epitopes as well. These results also raised questions on the differences in antibody recognition between HMW glutenin subunits and the other prolamins that may be due to quantitative or structural causes. As in the case of respiratory allergy to wheat, proteins from the albumin/globulin extract were also bound by IgE antibodies and there is a need to further characterize in these allergens specific albumin/globulin components

from cross-contaminating prolamins. However, the possibility for most wheat-allergic people to eat rice and maize safely indicates that the clinically relevant allergens are probably restricted to gliadins and glutenins.

Acknowledgements

The authors thank L. Parisot for handling of sera and V. Leduc for helpful discussions; F. Battais receives a grant from VANAM programme of region 'Pays de la Loire'.

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