

Different Profiles of Wheat Antigens Are Recognised by Patients Suffering from Coeliac Disease and IgE-Mediated Food Allergy

Claudia Constantin^a Wolf Dietrich Huber^b Gerhard Granditsch^b
Margit Weghofer^a Rudolf Valenta^a

^aDivision of Immunopathology, Department of Pathophysiology, Center of Physiology and Pathophysiology, and ^bDepartment of Pediatrics, Vienna General Hospital, Medical University of Vienna, Vienna, Austria

Key Words

Antigen profile · Coeliac disease · IgA · IgE ·
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Abstract

Background: Dietary intake of wheat can cause two distinct immunologically mediated diseases with severe gastrointestinal manifestations, coeliac disease (CD) and IgE-mediated food allergy. The pathomechanisms underlying these diseases are different, but the profile of the target antigens in wheat has not been compared for the two diseases. **Methods:** We compared IgA- and IgE-reactive antigens in wheat using sera from patients with coeliac disease (n = 35) and food allergy to wheat (n = 16) by one- and two-dimensional immunoblotting. Furthermore, the IgG subclass (IgG1–IgG4) reactivity to wheat antigens was studied by enzyme-linked immunosorbent assay. **Results:** IgA antibodies from CD patients and IgE antibodies from allergic patients recognised distinct profiles of wheat antigens. Furthermore, the IgG subclass responses to wheat antigens were different in CD and wheat-allergic patients. **Conclusion:** This study thus demonstrates that wheat contains antigens/epitopes which are preferentially recognised by CD patients, whereas others elicit IgE-mediated food allergy. This finding suggests that the nature of a food antigen may influence the quality of the pathological immune re-

sponse in the gut and has implications for the diagnosis and therapy of hypersensitivity to wheat.

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Introduction

Coeliac disease (CD) and IgE-mediated food allergy to wheat are considered as two immunologically mediated hypersensitivities of the gut with different underlying pathomechanisms. CD represents an enteropathy, which is caused by ingestion of wheat proteins in genetically predisposed individuals [1, 2]. CD patients exhibit IgA antibody and T-cell reactivity mainly against a complex fraction of alcohol-soluble proteins in wheat seeds, termed gliadins [3], and develop autoreactive IgA antibodies against several intestinal proteins, among them the major antigen, tissue-transglutaminase [4–6].

IgE-mediated food allergy, another genetically determined hypersensitivity disease of the gut, can also be caused by dietary intake of wheat proteins [7, 8]. While the pathomechanisms of CD [9] and wheat allergy [10] are relatively well described, less information is available about the antigens which are associated with these diseases and which represent targets for the humoral and cellular immune responses in patients. Fractionation of wheat proteins has been applied to identify protein subfractions with toxic activity in CD patients [11]. Further-

more, antibody reactivities have been analysed in CD patients [3, 12], but the molecular nature of the recognised antigens has not been studied in detail.

As far as wheat-induced food allergy is concerned, limited information is available about the disease-eliciting allergens. Food-allergic patients are reported to have IgE antibodies against the albumin and globulin fraction of wheat, against the α -, β - and γ -gliadins [13], ω -5 gliadin [14] and several less-characterised wheat components [15, 16].

However, no study has been performed to compare the IgA- and IgE-reactive wheat antigens recognised by CD and wheat-allergic patients, respectively.

In this study, we have used sera from CD patients and patients suffering from IgE-mediated wheat allergy to analyse the antibody reactivity to different protein fractions and to compare the profile of IgA- and IgE-reactive antigens by one- (1D) and two-dimensional (2D) immunoblotting. Furthermore, we studied IgA, IgE and IgG1–4 subclass reactivity to wheat proteins in the two patient groups by ELISA to obtain information about the composition of the IgG antibody response and the type of humoral immunity operative in the two diseases. Our findings that CD and wheat-allergic patients recognised mainly different antigen profiles and exhibited a different type of humoral immunity to wheat antigens indicate that despite a similar route of exposure, different types of wheat antigens/epitopes are recognised by CD and wheat-allergic patients.

Material and Methods

Patients

The study groups consisted of 35 patients with CD (10 males, 25 females: mean age 17 years, range 0.4–65 years; tables 1 and 2) and 16 wheat-allergic patients (10 males, 6 females: mean age 35 years, range 18–57 years; table 3). Serum samples from CD patients were analysed for anti-gliadin antibodies (IgA and IgG) by ELISA and IgA anti-endomysial antibodies were determined by indirect immunofluorescence using fixed cryostat sections of monkey oesophagus (INOVA, San Diego, Calif., USA). In almost all CD patients, detailed results of small bowel suction biopsies were available to confirm the diagnosis (table 1). In the CD patients studied, a typical flat mucosa was demonstrated, and histological analysis revealed villous atrophy, crypt hyperplasia and increased numbers of intraepithelial lymphocytes. Sera from patients with food allergy were analysed for total serum IgE levels and IgE specific for wheat, rye and allergen sources listed in table 3 (CAP-FEIA System; Pharmacia Diagnostics, Uppsala, Sweden). Clinical data (i.e. type of allergy) of these patients are summarized in table 3. CD patients were designated with Arabic numbers and allergic patients with roman numbers. For control purposes, sera from healthy individuals were included in all experiments.

Protein Extracts

SDS Extract. Three grams of wheat seeds were ground, dissolved in 32 ml of sample buffer [17] and boiled for 10 min. In order to remove insoluble particles, the extract was centrifuged at 10,000 g for 10 min at 4°C, and the supernatant was stored in aliquots at –20°C.

PBS Extract. In addition, a wheat protein extract was prepared by stirring 10 g of ground wheat seeds in phosphate-buffered saline (PBS) containing 1 mM phenylmethyl sulphonyl fluoride for 1 h at 4°C. After centrifugation (10,000 g, 10 min, 4°C), the protein concentration in the supernatant was determined using the Micro BCA-Protein Assay Reagent Kit (Pierce, Rockford, Ill., USA), and the extract was stored at –20°C until use.

NaCl Extract. For the extraction of the albumin/globulin fraction, 3 g wheat seeds were ground and extracted for 1 h at room temperature in 30 ml of 0.15 M NaCl buffer (10 ml/g). After centrifugation (10,000 g, 15 min, 8°C), the extraction step was repeated three times. Aliquots of the NaCl supernatants 1, 2 and 3 were analyzed [12].

EtOH Extract. The final pellet from the NaCl extraction was extracted in 30 ml of 70% (vol/vol) aqueous ethanol for 2 h at room temperature. After the extraction step, a clear supernatant was obtained by centrifugation at 10,000 g for 15 min at 8°C [12].

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and 1D Immunoblotting

Wheat protein extract was separated by a 14% preparative SDS polyacrylamide gel [17]. A protein molecular weight marker (Rainbow-Marker, Amersham Bioscience, Little Chalfont, UK) was used as a standard. After electrophoretic separation, proteins were either silver stained using the silver stain kit from Bio-Rad (Hercules, Calif., USA) or blotted onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) [18]. Sera for IgE detection were diluted 1:10, and for IgA detection 1:1,000 in buffer A (50 mM sodium phosphate buffer, pH 7.4, 0.5% w/v bovine serum albumin, BSA, 0.5% v/v Tween-20, 0.05% w/v NaN_3). Nitrocellulose strips were cut from the membranes and were blocked twice for 5 min and once for 30 min in buffer A at room temperature. Strips were probed overnight at 4°C with diluted patient sera. Control strips were incubated with serum from a healthy individual and with buffer without serum. Strips were washed three times for 30 min in buffer A. Bound human IgA was detected with a mouse monoclonal anti-human IgA antibody (M1265, CLB, Amsterdam, The Netherlands) which had been labeled with ^{125}I using the chloramine-T method [19]. Bound IgE antibodies were detected with ^{125}I -labeled anti-human IgE antibodies (Pharmacia). Blots were exposed to Kodak XOMAT films with intensifying screens (Kodak, Heidelberg, Germany) at –70°C.

Isoelectric Focusing and Two-Dimensional Immunoblotting

Wheat extract proteins were separated by 2D gel electrophoresis on the basis of charge in the first dimension using Immobiline Dry-Strips (pH 3–10 non-linear, 13 cm, Amersham Bioscience). PBS-extracted wheat proteins (600 μg) were lyophilised and then dissolved in 20 μl lysis buffer (8 M urea, 4% Triton X-100, 40 mM Tris, 20 mM dithiothreitol, DTT, and 0.057 mM phenylmethyl sulphonyl fluoride) and 250 μl rehydration buffer [8 M urea, 2% Triton X-100, a few grains of bromophenol blue, 0.057 mM phenylmethyl sulphonyl fluoride, 1% IPG buffer (Amersham Bioscience) and 4.5 mM DTT (Sigma, St. Louis, Mo., USA)] and applied to the

Table 1. Demographic, clinical and serological characteristics of CD patients

Patient	Age at biopsy years	Sex	Biopsy	EMA	Antigliadin antibodies				IgA Western blot	Diet (duration in days)
					IgA		IgG			
1	8	F	S	4+	126	+	86.5	+	+	no
2	19	F	S	3+	21.8	±	15.9	—	+	no
3	1	M	S	1–2+	121.9	+	188.7	+	+	no
4	3	F	S	4+	37.5	+	78.1	+	+	no
5	8	F	S	4+	113.4	+	142.8	+	++	no
6	1	M	S	4+	>200.0	+	>200.0	+	++	no
7	51	F	S	5+	>200.0	+	35	+	++	no
8	1	F	S	4+	>200.0	+	171	+	++	no
9	8	M	S	4+	0.39	+	0.28	—	±	no
10	15	F	S	3+	17.4	—	17.2	—	±	no
11	1	F	S	4+	0.21	±	0.322	±	±	no
12	1	M	S	1–2+	>200.0	+	193.1	+	++	no
13	2	F	S	3+	181	+	120	+	++	no
14	4	F	S	4+	>200.0	+	143.1	+	+	no
15	4	M	S	2+	55.5	+	104.3	+	±	no
16	32	F	S	4+	101.7	+	27	—	+	no
17	64	F	S	4+	28.9	—	0	—	±	no
18	2	F	S	4+	>200.0	+	145.5	+	++	no
19	32	F	P+S	2+	19.5	—	20.4	—	±	no
20	1	M	NA	1+	238.9	+	323.5	+	++	no
21	32	F	NA	5+	200.5	+	195	+	++	no
22	37	M	P	4+	66.2	+	35.1	+	±	yes (74)
23	19	F	S	3–4+	17.7	—	32.2	—	±	yes but many mistakes (200)
24	65	F	S	4+	109.8	+	76.4	+	+	yes but many mistakes
25	2	F	S	3–4+	223.2	+	23.5	—	+	yes but many mistakes (350)
26	6	F	S	4+	6.1	—	42.1	+	±	no
27	6	F	S	4+	5.9	—	9.4	—	±	no
28	46	F	NA	4+	18.8	—	23.7	—	±	no
29	16	F	S	1+	35.3	+	26.8	—	+	no
30	21	F	S	4+	39.2	+	3.7	—	±	no
31	38	M	NA	4+	301	+	25.1	—	++	no
32	2	F	S	4+	138.9	+	39.1	+	+	yes (21)
33	34	F	NA	4+	13	—	17.6	—	±	no
34	5	F	S	4+	178.3	+	333.6	+	++	no

F = Female; M = male; S = subtotal atrophy; P = partial atrophy; NA = not available; EMA = anti-endomysial antibodies; ++ = highly positive; + = positive; ± = borderline positive; — = negative. Results from other ELISA technology are in italics.

Table 2. Demographic, clinical and serological characterization of a boy with CD (patient 35) during periods of gliadin exposure and diet

Date of birth	Date of serum sampling	Duration of gliadin exposure, days	Diet days	Biopsy	EMA	Antigliadin antibodies				IgA Western blot
						IgA		IgG		
01.08.1996	08.01.1997	7	no	ND	2+	16.9	—	5	—	±
	05.03.1997	63	no	ND	3+	26.5	±	22.6	—	+
	07.04.1997	96	no	ND	3+	153.4	+	136.4	+	++
	26.05.1997	107	yes (38)	S	2+	0	—	55.6	+	—

S = Subtotal atrophy; EMA = anti-endomysial antibodies; ++ = highly positive; + = positive; ± = borderline positive; — = negative.

Table 3. Demographic, clinical and serological characteristics of allergic patients

Patient	Age at blood donation years	Sex	Food allergy	Asthma	RC	AD	Wheat IgE kUA/l	Rye IgE kUA/l	Total IgE kU/l	IgE Western blot
I	25	M	wheat intolerance	g,t,w	g,t,w,a	–	1.94	ND	154	++
II	57	F	wheat (OAS)	g,t	–	+	0.44	0.39	1,058	+
III	27	M	wheat (diarrhoea)	g,t	g,t,a,m	–	2.36	ND	657	++
IV	42	M	wheat (nausea)	–	g,a,m	–	–	0.59	95.4	+
V	30	F	wheat intolerance	g,t,a	g,t,a	+	1.2	1.74	277	+
VI	41	M	wheat intolerance	g,a	g,a	+	0.38	ND	>2,000	+
VII	42	F	wheat (OAS)	g,t	g,t,a	–	0.59	0.47	273	+
VIII	38	M	wheat intolerance	–	g,a	–	0.89	0.39	189	±
IX	18	M	wheat (dermatitis)	–	a	–	2.22	ND	>2,000	+
X	55	M	wheat (OAS)	–	t,a	+	0.43	<0.35	932	±
XI	35	M	wheat intolerance	t	t,g,a	–	1.11	1.54	453	++
XII	28	M	wheat intolerance	–	–	–	2.48	2.02	>2,000	++
XIII	34	F	wheat intolerance	g,t,a,fu,d	–	–	18.6	13.4	>2,000	++
XIV	29	F	wheat (RC)	a	a	–	2.83	1.45	1,035	±
XV	23	F	wheat intolerance	–	–	–	0.38	0.68	999	±
XVI	34	M	wheat (diarrhoea, flatulence)	–	g	–	3.1	3.08	155	++

F = Female; M = male; OAS = oral allergy syndrome; RC = rhinoconjunctivitis; AD = atopic dermatitis; g = grass pollen; t = tree pollen; w = weed pollen; m = mite; a = animal; fu = fungi; d = drugs; kUA/l = kilounits antigen per liter; ND = not done; ++ = highly positive; + = positive; ± = borderline positive; – = negative.

strips overnight at room temperature by in-gel rehydration. Then the strips were loaded onto a Multiphor II Electrophoresis unit (Amersham) and focused for 5 h at 200 V, 9 h at 1,000 V, 5 h at 2,000 V and 5 h at 3,500 V. The strips were placed in equilibration buffer (50 mM Tris-HCl, pH 8.8, 6 M urea, 30% v/v glycerol, 4% w/v SDS, a few grains of bromophenol blue and 65 mM DTT), once for 5 min and twice for 15 min, loaded onto 14% acrylamide gels and separated by SDS-PAGE; then proteins were blotted onto a nitrocellulose membrane.

ELISA

IgE, IgG1, IgG2, IgG3 and IgG4 binding to wheat protein extract was measured by ELISA. Plates (Nunc Maxisorb, Roskilde, Denmark) were coated with PBS-extracted wheat antigens (100 µg/ml) in PBS overnight at 4°C. The plates were washed twice with PBS, 0.05% v/v Tween-20 (PBST) and incubated for 2.5 h with PBST, 1% w/v BSA at room temperature. Patient sera were diluted 1:10 for the detection of specific IgE and 1:1,000 for the detection of IgG1, IgG2, IgG3 and IgG4 in PBST, 0.5% w/v BSA. The plates were incubated overnight at 4°C, washed five times with PBST and bound IgG1, IgG2, IgG3 and IgG4 were separately detected with monoclonal mouse anti-human IgG1–4 antibodies (PharMingen, San Diego, Calif., USA), diluted 1:1,000 in PBST, 0.5% w/v BSA overnight at 4°C. The specificity of the monoclonal anti-human IgG subclass antibodies had been confirmed by testing with purified human isotypes (Sigma). Then, plates were washed five times with PBST and incubated for 30 min at 37°C and 30 min at 4°C with a horseradish-peroxidase-coupled sheep anti-mouse antiserum (Amersham Bioscience) diluted 1:2,000 in PBST, 0.5% BSA. Plates were again washed five times with PBST and the colour reaction

was started by addition of 1.7 mM 2,2'-azino-bis-[3-ethyl-benzthiazoline-6-sulphonic acid] (Sigma) in 60 mM citric acid, 77 mM Na₂HPO₄·2H₂O and 3 mM H₂O₂. Plates were incubated in the dark, and extinctions (optical density, OD, 405 nm) were determined with an ELISA reader (Dynatech, Denkendorf, Germany). For IgE detection, the plates were washed five times with PBST and incubated for 1 h at 37°C and 1 h at 4°C with alkaline-phosphatase-labelled monoclonal mouse anti-human IgE (PharMingen) diluted 1:1,000 in PBST, 0.5% BSA. Again the plates were washed five times with PBST and incubated with alkaline phosphatase substrate (Sigma) in 13% diethanolamine, 4 mM NaN₃ (pH 9.8). Extinctions (OD 405 nm) were determined with an ELISA reader (Dynatech). All determinations were performed in triplicates, and results are displayed as mean values with an error of less than 10%.

Results

Different Extraction of IgA- and IgE-Reactive Antigens

Four different extraction procedures (PBS, SDS, NaCl and EtOH) were used for preparing wheat extracts. Silver staining of the different extracts (fig. 1a) showed that SDS buffer extracted most antigens, PBS and NaCl yielded comparable extracts and EtOH extraction gave a prominent band at 45 kDa (fig. 1a). Immunoblots showed that

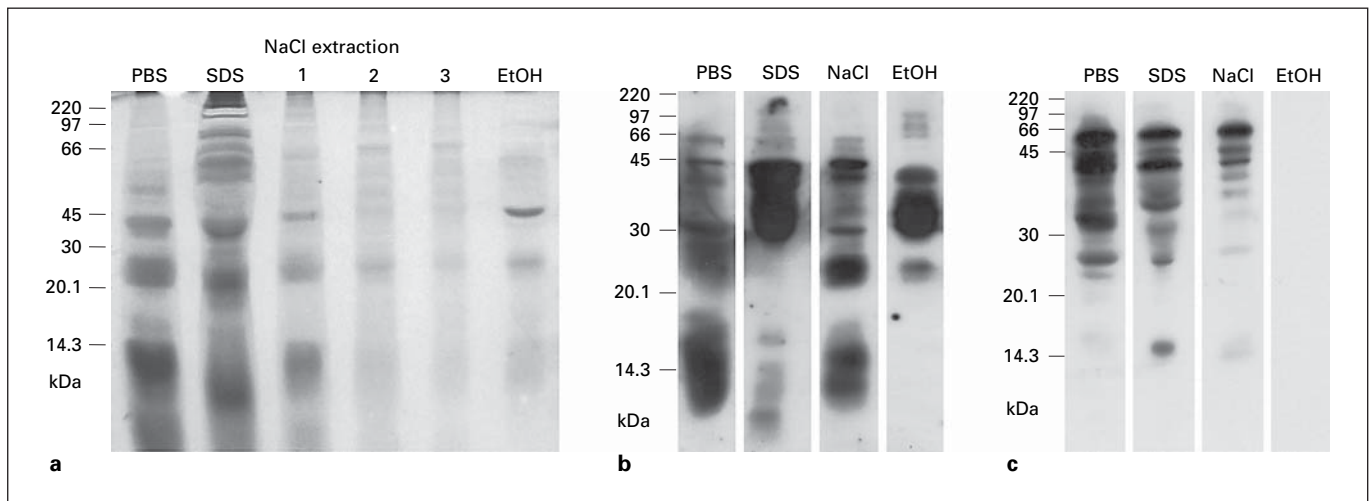


Fig. 1. Comparison of different wheat extraction procedures. Wheat proteins were extracted by PBS, SDS, NaCl and EtOH and visualized by silver staining of SDS-PAGE (**a**). IgA- and IgE-reactive antigens were detected by immunoblotting using sera from patient 6 (**b**) and patient XIII (**c**), respectively. Molecular weight markers (in kDa) are shown on the left.

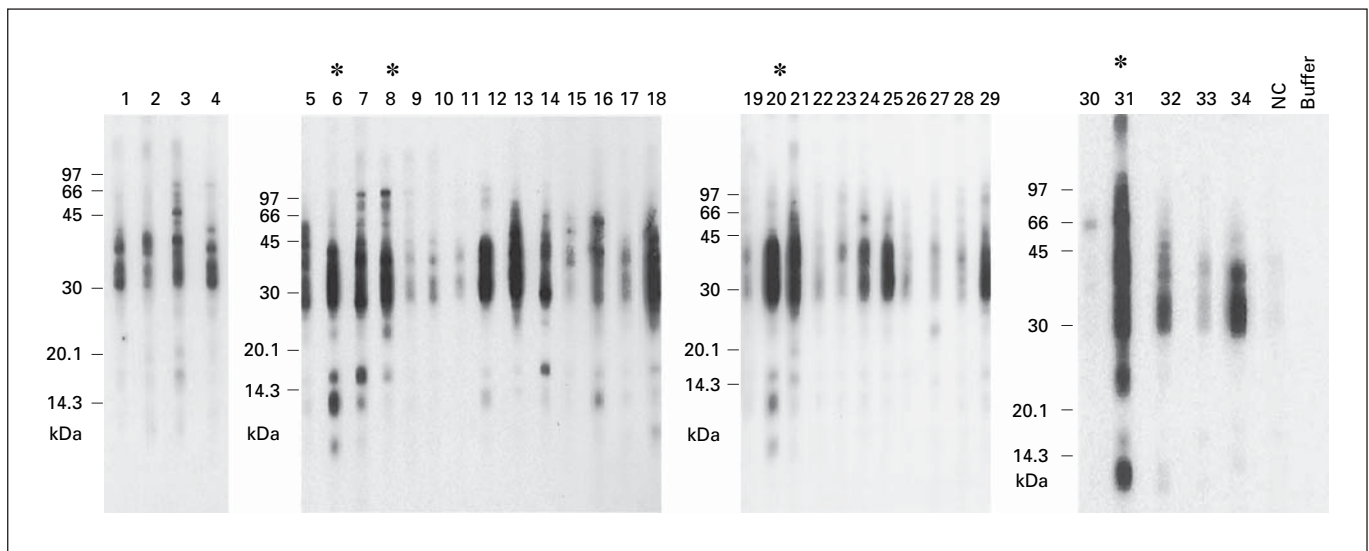


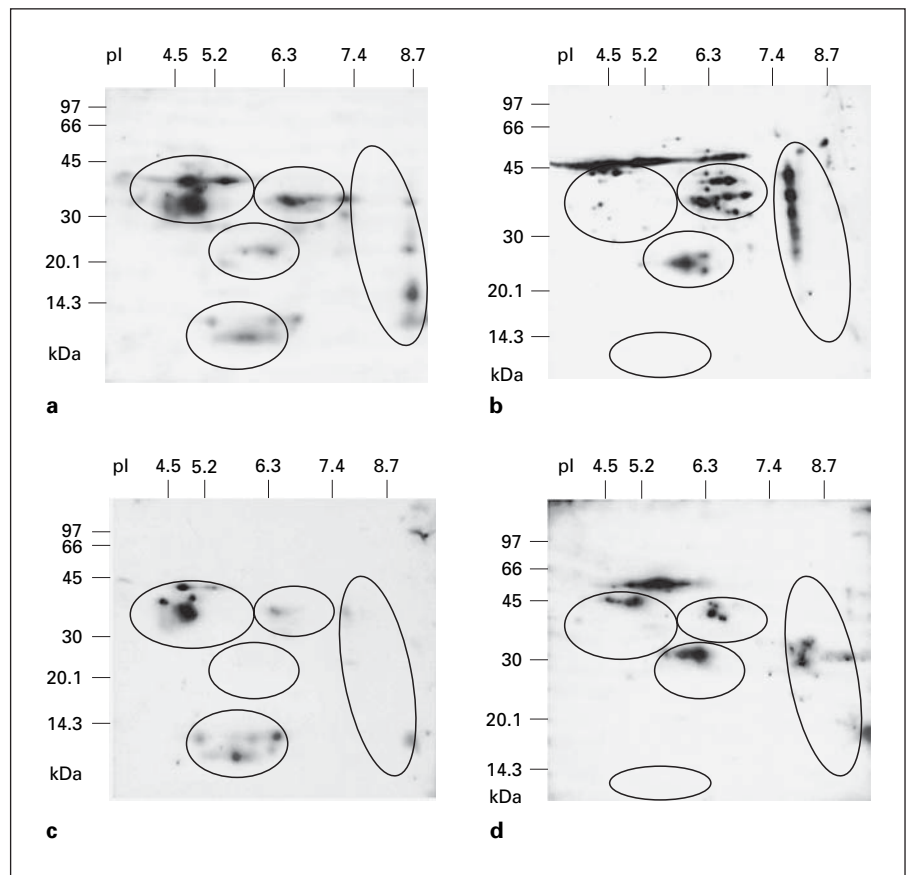
Fig. 2. IgA antibody reactivity of CD patients to 1D-separated wheat antigens. Nitrocellulose-blotted wheat extract was exposed to IgA antibodies from CD patients (1–34). For control purposes, serum from a healthy person (NC) and buffer without addition of serum (buffer) were included. Asterisks indicate sera analysed by 2D immunoblotting. Molecular weight markers are indicated on the left (in kDa).

IgA- and IgE-reactive wheat antigens were most efficiently detected in the SDS extract (fig. 1b, c). PBS and NaCl buffers resulted in the extraction of comparable IgA- and IgE-reactive antigens whereas in the ethanol extract only IgA- but no IgE-reactive antigens were found (fig. 1b, c).

IgA-Reactive Wheat Antigens Defined by 1D and 2D Immunoblotting Using Sera from CD Patients

For the characterisation of IgA-reactive wheat antigens, sera from 34 CD patients were used. Each of these patients exhibited elevated anti-gladin antibodies (IgA

Fig. 3. IgA reactivity of CD patients to 2D-separated wheat antigens. Sera from 4 CD patients (**a** patient 6; **b** patient 8; **c** patient 20, and **d** patient 31) with representative IgA reactivity to 1D-separated wheat antigens (fig. 1: asterisks) were probed for IgA reactivity to 2D-separated wheat extracts. Molecular weight markers (kDa) are indicated on the left and pI values on the top.



and IgG), as determined by ELISA, and showed positive anti-endomysial antibody reactivity (table 1). First we studied IgA reactivity of sera from CD patients to wheat antigens by 1D immunoblotting (fig. 2). IgA-defined antigens ranged mainly from 10–100 kDa and were grouped in three major clusters. The most frequently detected cluster of antigens ranged from 25–46 kDa and was recognised by all but one serum (No. 30). Eleven sera (3, 6–8, 12, 14, 16, 18, 20, 21 and 31) exhibited additional IgA reactivity to another antigen cluster in the range from 10–18 kDa. Third, antigens with a molecular weight of more than 45 kDa were detected by certain sera (e.g. 3, 5, 7, 8, 13, 16, 21, 24, 30 and 31).

For a detailed characterisation of the IgA-reactive antigens, 2D immunoblots were performed with sera from 4 patients with IgA reactivity to representative wheat antigens in 1D immunoblots and high levels of anti-gliadin IgA antibodies (table 1; fig. 2: patients 6, 8, 20 and 31). IgA reactivity to several antigens from 10–50 kDa with a pI from 4–9 was found (fig. 3). Most of the acidic antigens (pI 4–5.5) had a molecular weight from 30 to 45 kDa. Low-

molecular-weight wheat antigens (10–14 kDa) were detected in a pI range from 5 to 6.5 by patients 6 and 20. Patients 6, 8 and 31 reacted also with antigens between 25 and 32 kDa in this pI range. All 4 patients showed IgA reactivity to a group of spots between pI 6.3 and 7.4 in a molecular weight range from 30 to 44 kDa. Interestingly, we also found IgA reactivity to antigens in the neutral and basic range (pI 7.5–9) which exhibited greatly varying molecular weights (12–45 kDa). A 50-kDa antigen (pI 5–5.5) was detected by IgA antibodies from patients 8 and 31.

The Profile of IgA-Defined Wheat Antigens Remains Unaltered after Gluten Exposure

In order to monitor the profile of IgA-reactive antigens in response to gluten exposure early in life, serum samples from a 5-month-old CD child were collected. During gluten exposure, the levels of anti-gliadin antibodies and the anti-endomysial antibodies increased continuously. Already after 38 days of gluten-free diet, IgA anti-gliadin antibodies dropped beyond the detection level and anti-endomysial antibody reactivity decreased (table 2). The

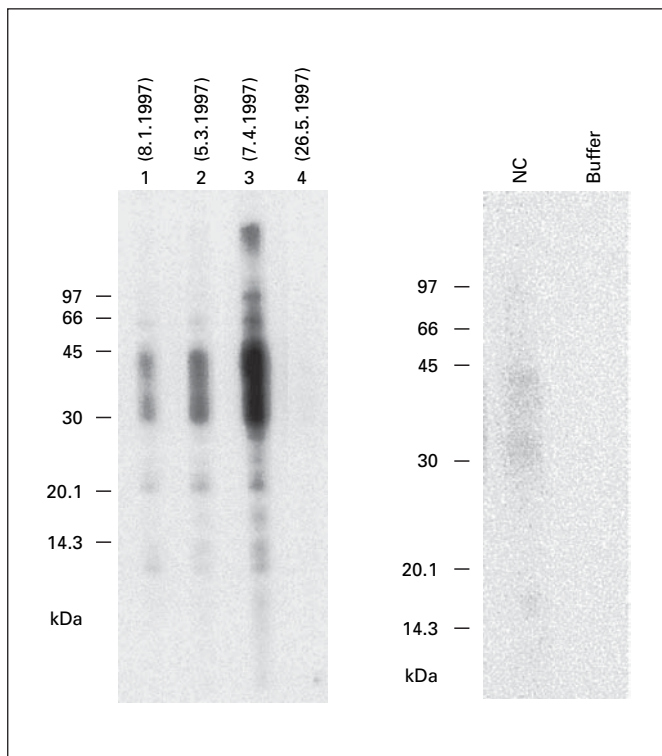


Fig. 4. Time course, profile and intensity of IgA reactivity to wheat antigens in a paediatric CD patient (35). Serum samples obtained after prolonged gliadin exposure (lanes 1–3) and after 38 days of gluten-free diet (lane 4; table 2) were exposed to nitrocellulose-blotted wheat antigens. Bound IgA antibodies were detected. Molecular weight markers (in kDa) are shown on the left. NC = Negative control.

analysis of IgA reactivity to nitrocellulose-blotted wheat antigens showed a continuous increase in IgA responses to the major antigen clusters which disappeared completely after 38 days of diet (fig. 4). While the intensity of IgA reactivity increased during prolonged gluten exposure, the profile of IgA-reactive antigens remained unaltered. Similar results were also observed for serum samples from other children (data not shown).

Characterisation of Wheat Allergens by 1D and 2D Immunoblotting Using IgE Antibodies from Wheat-Allergic Patients

Sera from wheat-allergic patients were analysed for IgE reactivity to 1D and 2D-separated wheat extract (fig. 5, 6). Serum IgE from allergic patients exhibited reactivity to a great variety of bands ranging from 10 to 70 kDa in 1D-separated wheat extract (fig. 5). Sera from 13 patients (I–III, V–IX, XI–XIV and XVI) reacted with allergens ranging from 10 to 15 kDa, and allergens in the range from 25 to 70 kDa were recognised by 15 of 16 sera. A detailed analysis of IgE-reactive wheat allergens was performed by 2D immunoblotting using serum from patient XIII (table 3) who had reacted with most allergens in the 1D immunoblots (fig. 6). Most of the antigens ranging between 25 and 66 kDa were acidic (pI 4–6.3). A few weakly IgE-reactive components with a molecular weight between 35 and 40 kDa were found at a pI of around 7.4 (fig. 6). Most of the low-molecular-weight allergens (around 15 kDa) had a pI of 6.3 or more.

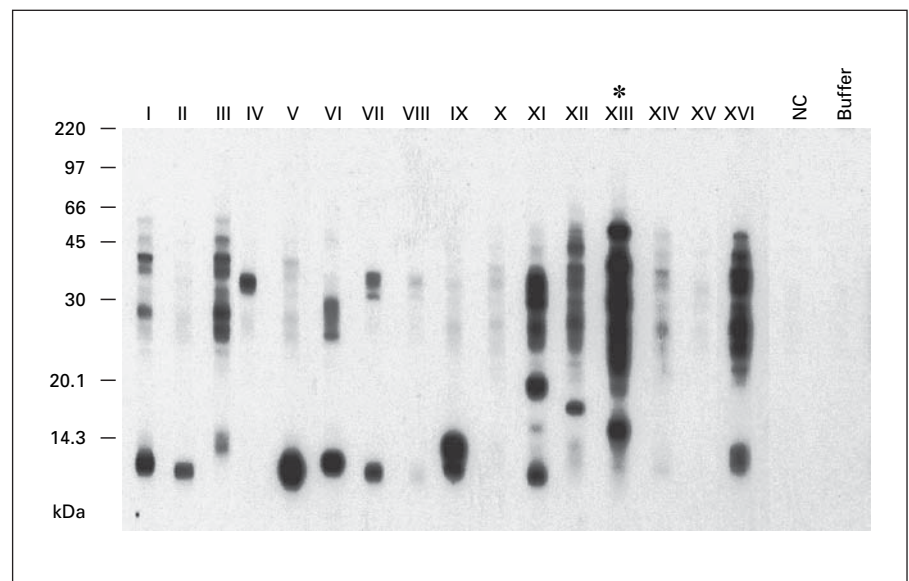


Fig. 5. IgE reactivity of allergic patients to 1D-separated wheat antigens. Nitrocellulose-blotted wheat extract was exposed to IgE antibodies from allergic patients (I–XVI). For control purposes, serum from a healthy person (NC) and buffer without addition of serum (buffer) were included. Serum XIII (asterisk) was analysed by 2D-immunoblotting. Molecular weight markers (in kDa) are shown on the left.

IgA Antibodies from CD Patients and IgE Antibodies from Allergic Patients Recognise Distinct Wheat Antigens

Although IgA antibodies from CD patients recognised certain antigens of similar molecular weights as those detected by IgE antibodies from wheat-allergic patients, striking differences were found. The reactivity profile of the CD patients was much more uniform than that observed among the allergic patients (fig. 2, 3). Furthermore, allergic patients reacted more frequently and pronounced with low-molecular-weight antigens (10–15 kDa) than the CD patients (fig. 2, 5). In 2D immunoblots, serum IgA from CD patients (fig. 3) and serum IgE from a representative wheat-allergic patient (fig. 6) recognised a great variety of spots ranging from 12 to 66 kDa (pI = 4–9). The main cluster of antigens recognised by IgA as well as by IgE was observed between 30 and 46 kDa and pI 4.5–5.2. However, several IgE-reactive spots were recognised specifically by wheat-allergic patients and are circled red in figure 6. They comprise clusters between 20 and 30 kDa (pI 4–4.5), low-molecular-weight spots (14–20 kDa) with a pI >6.0 and a 10-kDa acidic spot.

Wheat-Allergic Patients Lack Relevant IgA Reactivity to Wheat Antigens

Next we investigated if allergic patients also possess wheat-specific IgA antibodies. Compared to serum from a CD patient (20 in fig. 2), wheat-allergic patients showed weak (II, IV, X, XI and XVI) or no IgA reactivity to nitrocellulose-blotted wheat extract (fig. 7). The weak IgA

reactivity observed for some of the allergic patients showed however the same uniform pattern as that of CD patients (fig. 2, 7). Antigens between 25 and 46 kDa represented the uniform target for IgA antibodies.

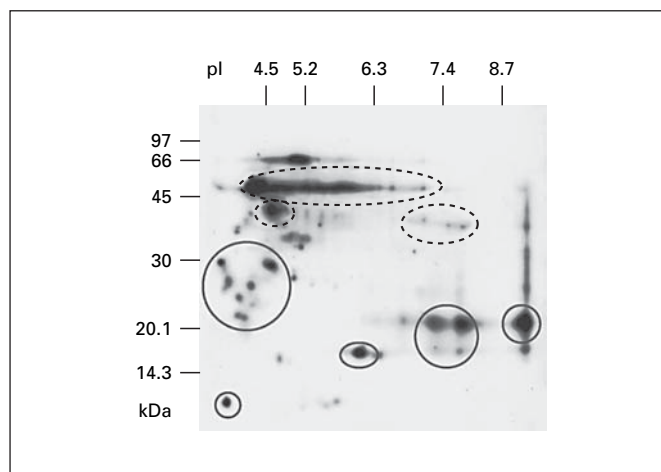
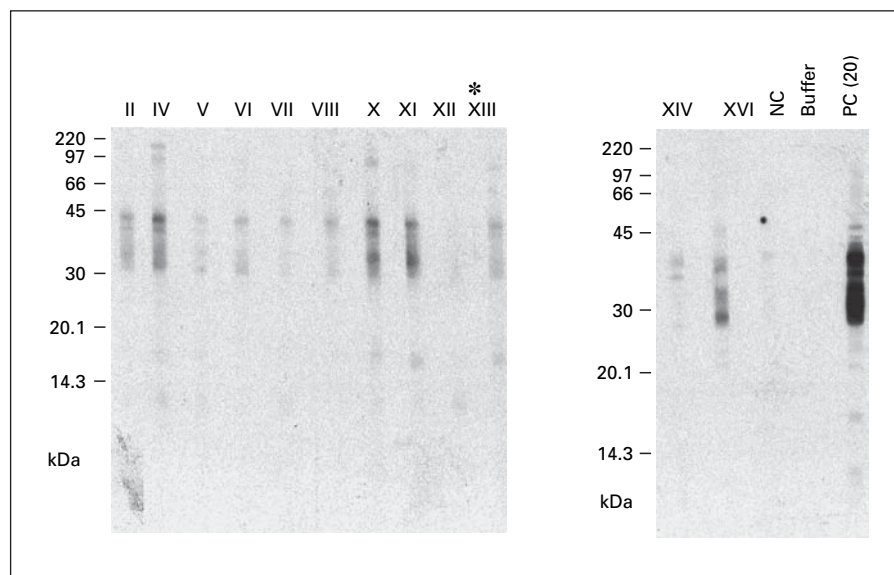


Fig. 6. IgE reactivity of a representative wheat-allergic patient to 2D-separated wheat antigens. Serum from a wheat-allergic patient (XIII) with high wheat-specific IgE levels and broad reactivity to 1D-separated wheat antigens (table 3; fig. 4: asterisk) was probed for IgE reactivity to 2D-separated wheat extracts. Specific IgE-reactive antigens (—) and antigens of a similar molecular weight and pI to those recognized by IgA from CD patients are indicated (---). Molecular weights (kDa) are indicated on the left and pI values on the top.

Fig. 7. IgA reactivity of allergic patients to 1D-separated wheat antigens. Nitrocellulose-blotted wheat extract was exposed to IgA antibodies from allergic patients (I–XVI). For control purposes, serum from a healthy person (NC) and buffer without addition of serum (buffer) were included. Serum from a CD patient (20) was included in the IgA blot of the allergic patients (PC). Molecular weight markers (in kDa) are shown on the left.



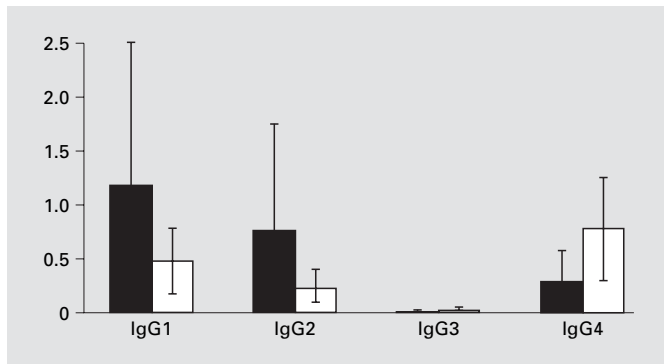


Fig. 8. IgG subclass reactivity to wheat antigens. IgG1–4 subclass reactivity was determined by ELISA for CD patients (n = 34; ■) and for allergic patients (n = 13; □). Mean OD values corresponding to the amount of antibodies bound are displayed on the y-axis.

Different IgG Subclass Reactivity to Wheat Antigens in CD and Wheat-Allergic Patients

Anti-wheat antibodies of the four IgG subclasses (IgG1–IgG4) were determined by ELISA for CD and wheat-allergic patients (fig. 8). A profound difference regarding IgG subclass reactivity was observed between the two patient groups. CD patients had higher IgG1 (mean OD: 1.153; SD: 1.273) and IgG2 (mean OD: 0.743; SD: 0.973) anti-wheat antibody levels than wheat-allergic patients. On the other hand, allergic patients exhibited higher IgG4 reactivity (mean OD: 0.756; SD: 0.464) than CD patients (mean OD: 0.279; SD: 0.285). Neither CD (mean OD: 0.011, SD: 0.01) nor wheat-allergic patients showed relevant IgG3 reactivity (mean OD: 0.016; SD: 0.019) to wheat antigens.

Discussion

Dietary intake of wheat can cause CD and IgE-mediated wheat allergy, which represent immunologically mediated hypersensitivity diseases with different underlying pathomechanisms [2, 7]. The differences in the immune responses to wheat antigens in CD and wheat allergy have been attributed to genetic factors which may affect either the balance of the immune response or the processing of antigens and the consecutive induction of the immune response [20, 21].

Our study provides evidence that wheat contains different antigens/epitopes which may preferentially be associated with – or even induce – CD or wheat allergy.

This assumption is supported by the comparison of IgA- and IgE-reactive wheat antigens determined by different protein extraction procedures in combination with 1D and 2D immunoblotting with sera from clinically well-characterised CD and wheat-allergic patients. In 1D immunoblots, CD patients showed a rather uniform IgA reactivity to wheat antigens, whereas wheat-allergic patients exhibited a rather heterogeneous IgE reactivity profile. These results were confirmed by 2D immunoblotting experiments showing that most of the IgA-reactive wheat antigens exhibited molecular weights and pI which were different from the IgE-reactive wheat antigens. The acidic IgA-reactive moieties of 30–60 and 10–14 kDa may correspond to gluten protein regions 1 and 3. The 25-kDa more basic antigens may match the gluten region 2 [22].

Furthermore, we found differences regarding the IgG subclass reactivities to wheat antigens between the two patient groups. Wheat-allergic patients showed a preferential IgG4 and IgG1 subclass reactivity to wheat antigens, which is indicative of a Th2 immune response [23]. By contrast, the IgG subclass reactivity of CD patients was characterized by a dominance of IgG1 and IgG2 antibodies. However, the humoral immune response of CD patients cannot be classified as being indicative of an isolated Th1 immune response because sera from CD patients frequently contained also IgG4 anti-wheat antibodies [24–27].

It is unlikely that the differences in IgA and IgE recognition of wheat antigens/epitopes in the two patient groups are due to differences in the route or dose of antigen exposure. Instead we suggest that wheat contains antigens/epitopes with an inherent capacity to induce either IgE or IgA responses. Based upon the genetic backgrounds of wheat-exposed individuals, these antigens/epitopes may be processed in a different manner and hence induce IgE or IgA responses of different magnitudes.

Similar to type I allergy [28], we found that the qualitative profile of IgA-reactive antigens remained unaltered during the natural course of CD and was also not affected by exposure to wheat. On the other hand, we found that the IgG subclass anti-wheat response in CD patients was highly heterogeneous. It is therefore possible that IgA-reactive, rather than IgG-reactive wheat antigens are involved in the pathogenesis of CD. To the best of our knowledge, our data are the first to indicate that, within a given antigen source, antigens/epitopes can induce different forms of immune responses (i.e. IgA vs. IgE responses). The molecular characterisation and identification of the wheat antigens by cDNA cloning and sequenc-

ing may therefore reveal antigenic features which initiate a certain type of immune response. In addition, it may provide us with defined molecular probes to study the pathogenesis of wheat-induced food allergy and CD. Finally, recombinant wheat antigens may be used for the diagnosis and perhaps treatment of the two diseases.

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