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Influence of the Allelic Variants Encoded at the *Gli-B1* Locus, Responsible for a Major Allergen of Wheat, on IgE Reactivity for Patients Suffering from Food Allergy to Wheat

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Wheat presents an important genetic diversity that could be useful to look for cultivars with reduced allergencity. ω_5-Gliadins have been described as major allergens for wheat allergic patients suffering from wheat-dependent exercise-induced anaphylaxis (WDEIA) and some cases of chronic urticaria (U). Our objective was to study the influence of genetic variability at the *Gli-B1* locus encoding for ω_5-gliadins on the reactivity of IgE antibodies from these patients. We selected cultivars expressing 13 alleles at *Gli-B1* including a wheat/rye translocation and studied the reactivity to gliadins of a rabbit antiserum specific for ω_5-gliadins and of IgE from 10 patients. The antiserum and IgE from nine patients with WDEIA and U strongly detected ω_5-gliadins expressed by most of the *Gli-B1* alleles but showed no or faint responses to the gliadins and secalins extracted from the translocated wheat. The selection of genotypes lacking the *Gli-B1* locus may reduce wheat allergenicity.

KEYWORDS: Wheat; food allergy; exercise induced anaphylaxis; ω_5-gliadins; allelic variants

INTRODUCTION

ω-Gliadins account for a minor percentage of prolamins (gliadins and glutenins), the major storage proteins of wheat. With the ω-secalins of rye and C-hordeins of barley, they belong to the group of S-poor prolamins characterized by a lack of sulfur and by sequences comprised of almost solely repeated motifs. ω-Gliadins can be divided into subgroups according to their amino acid composition, size, and electrophoretic mobility (*1*). Gliadins of the ω_5 type, which are the fastest moving components among ω-gliadins in acid-polyacrylamide gel elctrophoresis (PAGE), are characterized by the N-terminal sequence SRQ/LLSP and a particularly high Gln content (about 56% as compared to 43% for $\omega_{1,2}$) whereas $\omega_{1,2}$- or slow-moving ω-gliadins have sequences beginning with ARQ/ELNP or KELQS and a higher content in Pro (26% as compared to 20%) (*1*−*3*). Gliadin ω_5 migrate around 70 kDa in sodium dodecyl sulfate (SDS)-PAGE whereas $\omega_{1,2}$ are in a 45−65 kDa range.

Food allergy to wheat triggers various clinical manifestations as the atopic eczema/dermatitis syndrome, chronic urticaria, abdominal pain, and anaphylaxis. Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a particular form of anaphylaxis occurring mainly in adult patients that is induced by the combination of wheat consumption and exercise. Palosuo et al. (*4*) first demonstrated that a purified protein with the N-terminal sequence SRLL reacted strongly with IgE from WDEIA patients. It was named Tri a 19 according to allergen nomenclature. Further studies carried out with purified gliadin fractions confirmed that ω_5-gliadins were major allergens for patients suffering from WDEIA but also in some cases of chronic

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Table 1. Characteristics of Patients Whose Sera Were Used in the Study

patients	age (years)	symptoms	PIP[a] (mm)/ wheat flour	PIP (mm)/ rye	PIP (mm)/ ω-gliadins	DBPCFC[b] reactive dose wheat flour
3	65	chronic urticaria	6	ND[c]	ND	ND
31	37	WDEIA[d]	11.5	0.5	11	ND
45	50	chronic urticaria	3	2	2.5	10 g
48	32	anaphylaxis	2	ND	4	30 g
52	42	chronic urticaria	2	ND	7.5	440 mg
69	43	WDEIA	3	0	ND	30 g of flour + 3 g of gluten + 50 g of bread + exercise
80	49	WDEIA	3	2	4.5	ND
EBO32	53	WDEIA	5	7	ND	ND
EBO33	47	WDEIA	7	5	ND	ND
EBO36	32	WDEIA	28	ND	ND	ND

[a] PIP, prick in prick. [b] DBPCFC, double blind placebo control food challenge. [c] ND, not done. [d] WDEIA, wheat-dependent exercise-induced anaphylaxis.

urticaria (*5*−*7*). Immunodominant IgE-binding epitopes have been identified on this allergen for WDEIA patients (*8*, *9*).

Wheat cultivars and species present an important genetic variability originating from wheat polyploidy, extensive breeding, and hybridization between wheat and rye. This variability is exemplified by the important polymorphism of gliadins and glutenins as well as by strong variations in the amounts of these proteins in grains. Variations from 6.2 to 20% of total gliadins for total ω-gliadin content and from 4 to 100% of ω-gliadins for ω_5-gliadins were observed between wheat species and cultivars (*3*, *10*). ω_5-Gliadins from a single cultivar could be resolved into two reverse phase high-performance liquid chromatography (RP-HPLC) fractions, which were separated into three bands in SDS-PAGE and 10 polypeptides in two-dimensional PAGE (*2*). It is not known if this induces variability in the allergenicity of cultivars, as observed in the case of apple (*11*).

Although ω-gliadins are polymorphic mixtures, most are coded by the complex single loci *Gli-A1*, *Gli-B1*, and *Gli-D1* on short arms of the group 1 chromosomes (*12*). Small amounts are encoded by other loci on short arms of chromosomes 1A and 1B (*13*). The *Gli-A1* and *Gli-D1* loci were shown to encode ω-gliadins with N-terminal sequences beginning with ARQL and AREL (ω_2-gliadins) and other components with N-terminal sequence beginning with KEL (ω_1-gliadins—derived from the previous ones by post-translational cleavage) whereas the *Gli-B1* locus encoded the SRLL type (ω_5-gliadins) (*1*, *2*, *14*, *15*). In the 1BL/1RS translocated wheat, a part of the short arm of the 1B chromosome has been replaced with a portion of the short arm of the 1R chromosome of rye. This translocation, used to improve resistance to disease, consequently induced the loss of most ω_5-gliadins and the expression of secalins, i.e., mainly ω-secalins plus few proteins migrating in the γ- and β-prolamin zone.

Thus, contrarily to other gliadin components, the ω_5-gliadins seemed to mainly correspond to the ω-gliadin fraction encoded by the unique *Gli-B1* locus. It seemed, therefore, interesting to examine the impact on allergenicity of the polymorphism present at this locus. Sixteen different allelic variants have been reported at *Gli-B1* (*16*). The objective of our study was to explore the influence of the majority of this genetic variability at the *Gli-B1* locus, including the wheat/rye translocation, on IgE reactivity for patients suffering from WDEIA or chronic urticaria, patients for which ω5-gliadins are major allergens. These variants also allowed us to develop a better knowledge of ω_5 type gliadin isoforms.

Table 2. Allelic Composition of the 14 Wheat Cultivars at the *Gli-1* Loci

no. in the study	cultivar	*Gli-A1*	*Gli-B1*	*Gli-D1*
1	Chinese-Spring	a	a	b
2	Soissons	k	b	b
3	Prinqual	f	c	b
4	Chopin	f	d	b
5	Arsenal	f	e	b
6	Campremy	o	f	b
7	Feuvert	f	g	b
8	Rudi	o	h	b
9	Insignia	f	i	i
10	Ruso	m	m	b
11	Clément (translocated)	k	l	b
12	Pandas	a	m	b
13	Goya	o	q	b
14	Salmone	a	s	b

MATERIALS AND METHODS

Clinical Features of Patients. Sera were obtained from 10 adult patients with clinical symptoms of WDEIA (six cases), anaphylactic shock (one case), and chronic urticaria (three cases) (**Table 1**). Food allergy to wheat was established by clinicians by positive prick-in-prick tests (subcutaneous introduction in the epiderm of a small amount of native food) to wheat flour, gluten, wheat extract (Allerbio laboratory, Varennes en Argonne, France), or purified ω-gliadins and by standardized double blind placebo control food challenges with wheat flour, gluten, and bread (*8*). Some patients underwent tests that included physical activity: running for 5 min to 1 h after eating the triple combination wheat flour, gluten, and bread. Most of the selected sera reacted specifically with ω5-gliadins in enzyme-linked immunosorbent assay (ELISA) (*7*). Three sera from nonatopic subjects were used as controls.

The study was approved by the committee for protection of human subjects in biomedical research of Cochin Hospital (Paris). Patients gave their informed consent to the clinical procedure of diagnosis.

Wheat Samples. We selected 14 bread wheat cultivars (*Triticum aestivum*) expressing the 13 main alleles at the *Gli-B1* locus, encountered in world wheat collections, and, respectively, five and two different alleles at the *Gli-A1* and *Gli-D1* loci (**Table 2**). These 13 alleles included the *Gli-B1l* allele corresponding to the 1BL/1RS translocation found in the wheat cultivar Clément.

Protein Extraction and Electrophoresis. Gliadins were extracted from flour, using a sequential procedure, and then precipitated with glacial acetone, dehydrated, and stored at −80 °C before use. The albumin−globulin fraction was first removed according to the procedure described by Marion et al. (*17*). Gliadins were extracted in 50% (v/v) propanol according to Singh et al. (*18*). For electrophoresis, the gliadin fraction was neither reduced nor alkylated and 200 μL of the gliadin solution was finally added with 100 μL of the electrophoresis solution containing 2% w/v SDS, 40% (v/v) glycerol, and 8% (v/v) 1 M Tris

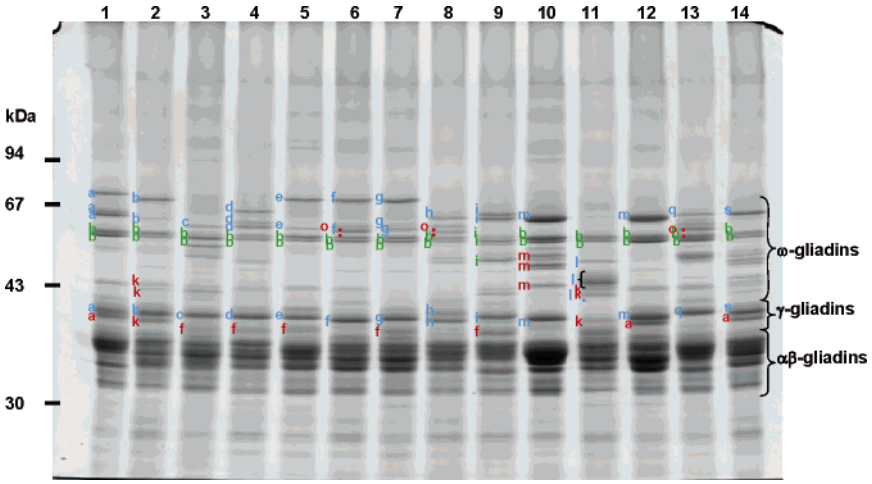

Figure 1. Separation by SDS-PAGE of gliadins extracted from the different wheat cultivars—Allelic variants encoded at the *Gli-B1*, *Gli-A1*, and *Gli-D1* loci are identified by blue, red, and green letters, respectively. The numbers assigned to the cultivars were those of **Table 2**.

HCl buffer, pH 8.0. The SDS-PAGE of gliadins as well as the allelic identification of the mobility bands were performed according to Branlard et al. (*19*). Briefly, chromosomal assignments were made using aneuploid lines where a pair of chromosomes was lacking (nullisomic lines) or a fragment of the chromosome arm was deleted (such as telosomic lines).

Analysis of Serum Reactivity by Immunoblotting. After SDS-PAGE, gliadins from the 14 cultivars were electroblotted to nitrocellulose membranes (0.2 μm, Sartorius Germany) as described by Laurière (*20*). Semidry transfer was achieved at 300 mA during 30 min for a 140 mm × 130mm × 1 mm gel size.

Membranes were incubated for 4 h in a blocking solution of 4% polyvinylpyrrolidone (PVP-40, Sigma P0930) in phosphate-buffered saline Tween 20 0.1% (PBS/T). They were washed three times with PBS/T containing 2% PVP, and patient sera diluted 1/20 in the washing buffer were added overnight. Each step was performed at room temperature under gentle agitation. After four washes, anti-human IgE antibodies (ϵ chain) labeled with peroxidase (P0295, Dako, Denmark) were added diluted 1/100000 in the washing buffer for 1 h. After further washing, membranes were incubated in a chemiluminescent substrate (SuperSignal West Dura Extended Duration Substrate, 34076, Pierce, United States) for 5 min without agitation, and then, luminescence was detected on a Kodak X-OMAT AR-5 film (Pharmacia 165 1496) after exposure for 30 min.

We also studied the reactivity of rabbit polyclonal anti-peptide antibodies (anti-NT2-ω) directed against the N-terminal sequence of ω_5-gliadins (peptide SRLLSPRGKELGC). These antibodies recognized specifically gliadins of ω_5 type (*15*). The anti-serum was diluted 1/5000, and the immunoblotting procedure was carried out as previously described.

Determination of ω5-Gliadin Proportion by RP-HPLC. The proportion of ω_5-gliadins according to total gliadins was determined by RP-HPLC. Gliadins were extracted from flour in 50% (v/v) ethanol after removal of the albumin/globulin fraction (*17*). After dialysis and lyophilization, gliadins were dissolved at 1 mg/mL in 0.1% (v/v) TFA and 25% (v/v) acetonitrile. Samples were analyzed on a C18 column (Nucleosil 300 Å, 5 μm, Macherey-Nagel). Elution was performed at 1 mL/min with a gradient formed from solvent A [0.1% (v/v) TFA in water] and B [0.08% TFA, 99% acetonitrile]: 0−5 min 25% B, 5−55 min 25−54.7% B. The injection volume was 100 μL. Protein was detected at 214 nm. A purified ω_5-gliadin sample (*7*) was used as reference.

RESULTS

Description of Gliadin Components Encoded at the *Gli-B1*, *Gli-A1*, and *Gli-D1* Loci. Gliadin components encoded by the different allelic variants at the *Gli-B1*, *Gli-A1*, and *Gli-D1* loci were visualized after SDS-PAGE separation (**Figure 1**—

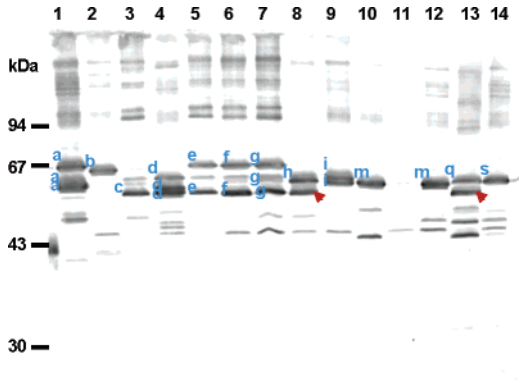

Figure 2. Reactivity in immunoblotting of polyclonal antibodies raised against the N-terminal peptide of ω_5-gliadins (anti-NT2-ω) with the gliadins extracted from the different wheat cultivars. Allelic variants encoded at the *Gli-B1* locus are identified by blue letters.

bands identified, respectively, by blue, red, and green letters). They included ω-gliadins (migration between 43 and 70 kDa) as well as some γ-gliadins (around 40 kDa) and $\alpha\beta$-gliadins (30−35 kDa—not annotated for correspondence to loci). Twelve out of the 13 allelic groups encoded at *Gli-B1* expressed 1−3 ω-gliadin bands with M_r values ranging from 63 to 70 kDa. Two very faint bands were observed in this mobility zone for the 1BL/1RS translocated cultivar Clément possessing the *Gli-B1l* allele, which codes for several bands of ω-secalins migrating between 45 and 55 kDa. ω-Gliadins of lower M_r (bands around 60 kDa) were present in all of the cultivars and corresponded to alleles b or i encoded at the *Gli-D1* locus. ω-Gliadins expressed by the *Gli-A1* locus are smaller than 1B- or 1D-encoded ω-gliadins (*14*). Some of them were annotated on the gel (allelic variants o, m, and k).

Reactivity of the Anti-NT2-ω Antibodies with the Gliadins Extracted from the Different Wheat Cultivars (Figure 2). The rabbit anti-NT2-ω antiserum, raised against the N-terminal peptide SRLLSPRGKEL, specific for ω_5-gliadins, reacted, with a strong intensity, with all of the ω-gliadin bands encoded by 12 *Gli-B1* alleles (a−i, m, q, and s), which allowed identification of ω_5-gliadin bands. Strongly detected as well in cultivars Rudi and Goya (**Figure 2**, nos. 8 and 13) was a 63 kDa band (arrow), indicating that some ω_5-gliadin components had not been annotated on the gel. Only two very faint bands (at 65 and 45 kDa) were detected in the gliadin extract of the translocated cultivar Clément. The antiserum did not react with ω-gliadins

Table 3. Proportion of ω_5-Gliadins According to Total Gliadins in the Wheat Cultivars

no. of cultivars	cultivar	ω_5/total gliadins
1	Chinese-Spring	4.8%
2	Soissons	ND
3	Prinqual	1.2%
4	Chopin	6.3%
5	Arsenal	ND
6	Campremy	4.4%
7	Feuvert	ND
8	Rudi	7.8%
9	Insignia	4.5%
10	Ruso	3.3%
11	Clément (translocated)	1.6%
12	Pandas	2.9%
13	Goya	7%
14	Salmone	4.8%

encoded by the *Gli-D1* or the *Gli-A1* alleles nor with $\alpha\beta$- and γ-gliadin bands. The anti-NT2-ω serum also weakly detected several minor bands of high mobility (43−55 kDa, not annotated) in the ω-gliadin migration zone. Staining was also observed in a migration zone of M_r higher than 100 kDa. This immunochemical response corresponded to aggregated ω-gliadin components present in very low amounts in gliadin extracts and hardly visible on the gel. They may correspond to a minor fraction of ω-gliadins possessing a cystein in their sequence leading to their incorporation among the glutenin subunits (*21*, *22*).

ω5-Gliadin Content of Wheat Cultivars. The percentage of ω5-gliadins related to total gliadins was determined for 11 of the 14 cultivars by RP-HPLC (**Table 3**). Cultivar Prinqual (no. 3) displayed a very low ω_5-gliadin content corresponding to a band of weak intensity on the Coomassie stained gel. Although two harldly visible ω_5-gliadin bands were observed for cultivar Clément (**Figure 1**, no. 11) on the gel, a low amount of proteins was eluted from the column in the ω_5-gliadin retention times. Similar results were obtained by Wieser et al. (*10*) for this cultivar indicating that a small amount of other gliadins or secalins might have the same hydrophobicity. Other wheat cultivars contained proportions of ω_5-gliadins ranging from 2.9 to 7.8% of total gliadins. These proportions were roughly related to the number of ω5-gliadin bands detected by the anti-NT2-ω antiserum (**Figure 2**): Two or three bands were recognized in cultivars 1, 4, 6, 8, 9, and 13 as having the highest ω_5-gliadin relative amounts (4.4−7.8%) and a single band in cultivars 10, 12, and 14 with lower ω_5-gliadin amounts (2.9−4.8%).

Reactivity of Patient IgE with the Different Gliadin Extracts (Figure 3). Bands corresponding to ω_5-gliadins expressed by 10 (alleles a, b, d−g, i, m, q, and s) of the 13 alleles encoded at *Gli-B1* locus were detected by IgE from all of the sera. The intensity of responses varied according to sera. The band corresponding to the *Gli-B1c* allele (cultivar Prinqual, no. 3) was detected with a low intensity by eight sera and not at all by two sera (for example, **Figure 3b**). Three sera reacted only weakly with the band corresponding to *Gli-B1h* allele (cultivar Rudi no. 8; for example, **Figure 3b**). IgE from nine of the 10 sera showed faint (**Figure 3c**) or no response (**Figure 3a**,**b**) to gliadins and secalins extracted from the translocated cultivar Clément (no. 11). The patient with anaphylaxis (sera 48−**Figure 3d**) had IgE reacting with a 44 kDa band (arrow), not detected by anti-NT2-ω antibodies, and corresponding to an 1RS-encoded ω-secalin. The low reactivity of patient IgE toward cultivars Prinqual and Clément is most probably due to the lack or low amount of ω_5-gliadins. The low reactivity to cultivar Rudi is particular to a few patients and does not correspond to a low ω_5-gliadin content nor to a specific patient symptom.

Patient IgE reacted practically solely with ω_5-gliadin bands. ω-Gliadins encoded by the *Gli-D1b* allele were never detected. One band of similar mobility or identical to the fastest band encoded by the *Gli-D1i* allele (cultivar Insignia no. 9) was recognized by seven sera (for example, **Figure 3a**). None of the annotated *Gli-A1* bands were bound by patient IgE. As the polyclonal antiserum, some patient sera detected minor bands (between 43 and 55 kDa) of the ω-gliadin zone. The sera 52 (additional figure) and 48 (**Figure 3d**), having the strongest intensity of IgE response, also detected weakly some bands among the γ- and $\alpha\beta$-gliadins. For these two sera, staining was also observed for agreggated high M_r components as for the anti-NT2-ω antibodies.

The reactivity of sera from nonatopic subjects was controlled on gliadins extracted from two cultivars (Soissons and Clément). The three sera did not detect any protein from these extracts.

DISCUSSION

This work was carried out on a collection of wheat cultivars possessing 13 different alleles at the *Gli-B1* locus including the *Gli-B1l* allele characteristic of the 1BL/1RS translocation. These 13 different alleles are all of the alleles that were found at the *Gli-B1* locus in a collection of 200 French and European cultivars (*19*). They comprised the most common alleles of European wheat (alleles b, f, and l encountered in 24.5, 46.5, and 6.5% of the analyzed bread wheat cultivars) as well as rare ones (alleles a, c, i, and s each found in one cultivar). This representative collection was used to analyze the reactivity to gliadins of IgE from wheat allergic patients suffering from WDEIA, chronic urticaria, or anaphylaxis.

The anti-NT2-ω antiserum was raised against the peptide "SRLLSPRGKEL" corresponding to the N-terminal sequence of ω-gliadins described as ω_5 type by Kasarda et al. (*1*). It specifically recognized these gliadins and did not react in ELISA with $\alpha\beta$-, γ-gliadins, glutenin subunits, or with $\omega_{1,2}$-gliadins (*15*). In Western blotting, this antiserum strongly detected the ω-gliadin bands encoded by all of the *Gli-B1* alleles, except allele l encoding for secalins instead of ω5-gliadins, and did not react with those encoded by *Gli-D1* and *Gli-A1* nor with other gliadins. Single mutations in positions 1 and 3 of the N-terminal sequence were observed for ω5-gliadins from a few bread wheat cultivars (*2*, *3*). Our study showed that no significant variation seemed to exist between the N-terminal sequences of ω-gliadins expressed by the different *Gli-B1* alleles. Our work confirmed thus on a larger wheat collection the criteria used for the denomination of ω_5-gliadins (*1*−*3*): They are ω-gliadins with the SRLL type N-terminal sequence (with high M_r: 60−70 kDa) and expressed almost exclusively by the unique *Gli-B1* locus.

The anti-NT2-ω antiserum also reacted very weakly, in extracts from all cultivars, with 1−4 bands between 43 and 55 kDa, that were not or hardly visible on the Coomassie stained gel. These bands may correspond to truncated ω_5-gliadins expressed in very low amounts. As a faint 45 kDa band is detected by the antiserum in cultivar Clément, these bands may be encoded at the *Gli-B1* locus with a low amount of residual *Gli-B1* expression in the translocated cultivar or may correspond to minor bands encoded by other loci. Anti-NT2-ω antibodies also detected in all cultivars, except Clément, high M_r aggregates. They may correspond to modified ω-gliadin com-

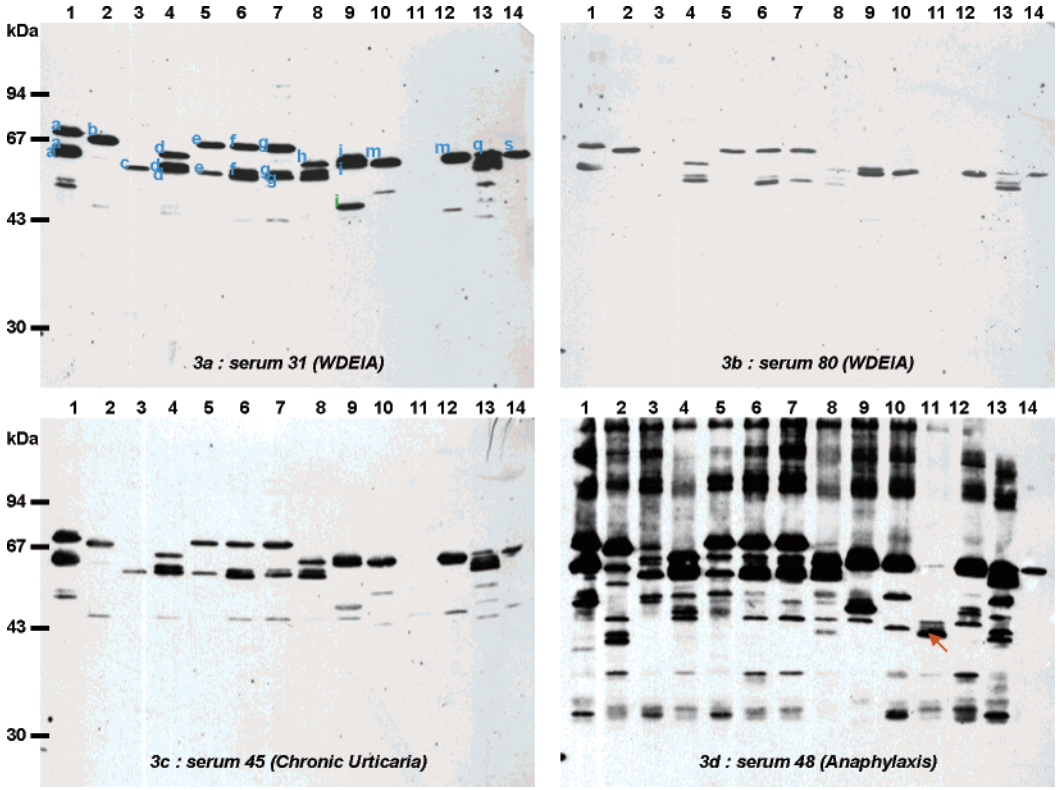

Figure 3. Reactivity in immunoblotting of IgE from patients with WDEIA (**a**,**b**), chronic urticaria (**c**), or anaphylaxis (**d**) with the gliadins extracted from the different wheat cultivars. Allelic varients encoded at the *Gli-B1* locus are identified by blue letters.

ponents (also called D type glutenin subunits) that have acquired a cystein residue and a mobility of glutenins (*22*). At the moment, only modified ω-gliadins with N-terminal sequence beginning with ARQL or KEL have been identified (*21*, *23*). This work indicated that low amounts of SRL type ω-gliadins encoded by *Gli-B1* may also be incorporated into glutenin aggregates. The presence of these ω-gliadins in high M_r fractions might also be a consequence of some other cross-linkage than disulfide cross-linkage.

IgE from patients with WDEIA, chronic urticaria, and anaphylaxis strongly reacted with 10 of the 13 *Gli-B1* alleles. None of them, except serum from the patient with anaphylaxis, reacted significantly with the cultivar Clément. Hence, IgE from the patients with WDEIA and chronic urticaria selected in this study did not react with the ω-secalins or γ-secalins expressed by the short arm of chromosome 1R of rye, although reactivity has been described to γ35- and γ75-secalins for patients with WDEIA (*24*, *25*). For these patients, IgE reactivity to gliadins and secalins differs from the cross-reactivity between these proteins observed for IgA of celiac patients.

The intensity of IgE responses was lower with cultivar Prinqual, which expressed a very low amount of ω_5-gliadins. It might also be that proteins expressed by *Gli-B1c* differed in epitopic sequences or missed some of the epitopes bound by IgE.

The reactivity of patient IgE and that of anti-NT2-ω polyclonal antibodies specific for ω_5-gliadin N-terminal sequence was quite similar toward the main ω_5-gliadin components; a few sera also reacted, as the polyclonal antiserum did, to ω_5-gliadin like components of lower or higher M_r. As compared to the group of patient sera, the antiserum did not detect any additonal components. Epitopes bound by IgE from patients with WDEIA have been localized in the repetitive domain of ω_5-gliadins (*8*, *9*, *26*). Epitopes bound by some of the sera included in this study (sera 3, 31, 45, and 52) were determined and corresponded to known immunodominant epitopes (QQIPQQQ; QQFPQQQ or QQLPQQQ (*8*); and Denery-Papini, personnal communication). There is thus a close association between the N-terminal SRLL sequence and these epitopes. Indeed, peptides QQIPQQQ, QQFPQQQ, and QQLPQQQ appeared quite specific for the ω_5-gliadins; they cannot be found 100% identical in the published sequences of ω_2-gliadin (AAG17702) nor in that of ω-secalin (AAB58403), whereas they are repeated, respectively, four, 23, and four times in the ω_5-gliadin sequence (BAE20328). The highest identity scores with ω_2 sequence were obtained with hexapeptides (not heptapeptides) comprising at least one mutated amino acid (sequence alignment with www.expasy.ch/tools/sim-prot.html). Assuming that the published sequences of ω-gliadins are well-representative of all ω-gliadins expressed in the studied varieties, this suggests that a very limited change in size or the sequence of the allergenic motif is sufficient to hinder the reactivity with patient IgE. These results are a strong indication that it would be possible to effectively eliminate patient response to ω_5-gliadins without altering the whole gliadin composition but only the proteins encoded at the *Gli-B1* locus.

Several attempts to abolish patient reactivity to wheat were made by using enzymatic or chemical treatments (*27*−*30*). However, it seems that no concrete applications have emerged at the present time. The main reasons may be the loss of flour bread-making properties and the production of new epitopes capable of inducing severe symptoms in wheat tolerant individuals (*31*, *32*). The screening of wheat cultivar collection represents an interesting approach among strategies aiming at decreasing the allergenicity of wheat-based products. Differences in allergen recognition among seven wheat cultivars have already been observed for patients with baker's asthma (*33*). A screening performed on 321 wheat cultivars also revealed important variations in their recognition by a unique patient serum possessing IgE binding to gliadins, glutenins, and

α-amylase inhibitors (*34*). In the case of celiac disease, large variations were also detected in the amount of T-cell stimulatory sequences present in several wheat species and varieties (*35*). This present work showed the existence of wheat cultivars with a reduced content in ω_5-gliadins or lacking this allergen due to translocation with 1RS chromosome arm from rye. Many countries have selected cultivars having the 1BL/1RS translocation, but those cultivars were either often discarded due to their poor bread-making properties or merely blended with normal wheat for bread making. Our results have shown that geneticists could make further progress in selecting wheat lacking the *Gli-B1* locus for future cultivars specifically oriented to patients with food allergy to wheat. Because the numerous genes clustered at the *Gli* loci have similar sequences, the silencing technology using siRNA may lead to the nonexpression of the majority of ω-gliadins in genetically modified wheat. Considering that the *Gli-B1* locus is located on a satellite at the distal end of the short arm of chromosome 1B, breeders could have quicker results in selecting genotypes having deleted the 1BS satellite.

In conclusion, IgE from WDEIA or chronic urticaria patients displayed a strong and specific reactivity to ω_5-gliadin isoforms encoded by most of the *Gli-B1* alleles. On the contrary, these antibodies yielded hardly any reaction to gliadins and secalins of a 1BL/1RS translocated wheat. Because ω_5-gliadin genes are clustered at the *Gli-B1* locus, the selection of genotypes lacking the *Gli-B1* locus thus represents an effective genetic way to eliminate ω_5-gliadin type sequences as well as IgE reactivity to gliadin extract for wheat allergic patients with symptoms of WDEIA and some cases of chronic urticaria. This study also showed that no significant variation exists between ω_5-gliadin isoforms either in their N-terminal sequences or in repeated domains containing IgE-binding epitopes.

Supporting Information Available: Figure giving other examples of patient sera reactivity: Reactivity in immunoblotting of IgE from patients with WDEIA and chronic urticaria with the gliadins extracted from the different wheat cultivars. This material is available free of charge via the Internet at http://pubs.acs.org.

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