

Original article

Genetic differences in omega-gliadins involved in two different immediate food hypersensitivities to wheat

Background: Anti-gliadin IgE are expressed in patients with food allergy associated to skin immediate hypersensitivity to hydrolyzed wheat proteins (IHHWP). It is not known if they react with ω 5-gliadins, the major allergens in wheat dependant exercise-induced food anaphylaxis (WDEIA), encoded on wheat chromosomes 1B.

Methods: Unmodified gliadins from 14 wheat varieties expressing most of the 1B ω -gliadin alleles, were immunoprobed after SDS-PAGE and blotting, with four sera from patients with IHHWP, and two with WDEIA. Gliadins reacting with IgE were visualized using chemiluminescence and identified according to their mobility and typical SDS-PAGE pattern. The resulting signal was also measured to compare their IgE reactivity.

Results: IHHWP and WDEIA sera exhibited distinct patterns of reactivity. IgE of patients with IHHWP reacted mainly with all ω -gliadins alleles and one γ -gliadin encoded respectively on chromosomes 1D and 1B, but not with any ω 5-gliadins alleles as for WDEIA. A few other reactive alleles of ω -gliadins were encoded on chromosomes 1A. Unassigned additional bands of the whole gliadin pattern were also reactive. The four patients with IHHWP exhibited almost the same pattern of reactivity. Main differences concerned band reactivity which modulated the overall reactivity of each wheat variety.

Conclusions: The IgE epitopes involved in IHHWP and WDEIA are different. This suggests that the protein state and the route of exposure to very similar gluten structures, probably orientate the pattern of epitope reactivity and the wheat food allergy manifestations.

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Hydrolyzed wheat proteins (HWP) are now common food and nonfood ingredients. For example, they can be used in cosmetics. Immediate hypersensitivities to these ingredients have already been described (1–3). We recently reported nine patients with immediate contact urticaria triggered by HWP present in cosmetics, mainly body moisturizing creams (4). Six of them exhibited also immediate food allergy when eating foods containing HWP, although they can eat normal bread, pasta and pastries. We found that IgE from all these patients

reacted with epitopes already present in native wheat proteins. All patients reacted with salt-soluble proteins (albumins and globulins). Furthermore, serum IgE reacting with gliadins was observed only in patients with associated food allergy. Gliadins are with glutenins, storage proteins that associate to make gluten. They are highly polymorphic. They are divided into α/β -, γ - and ω -gliadins, according to their decreasing acid-PAGE mobility. Most of them are encoded in bread wheat at six main loci on the short arms of the first (*Gli-A1*, *Gli-B1*, *Gli-D1*), and the sixth (*Gli-A2*, *Gli-B2*, *Gli-D2*), homoeologous chromosomes groups (5). They are encoded as multigenic families expressed constitutively and exclusively in the grain endosperm which gives the wheat flour. These loci are inherited all together as Mendelian units, forming multiallelic groups. Each allelic group produces a typical banding pattern using either acid-PAGE or SDS-PAGE, which is the basis of variety identification. The different allelic groups

Abbreviations: HWP, hydrolyzed wheat proteins; IHHWP, immediate hypersensitivity to HWP; WDEIA, wheat dependant exercise-induced anaphylaxis; *Mr*, relative molecular mass; *Gli-A1 m*, *Gli-B1a*, *Gli-D1b*, nomenclature codification of gliadin multialleles encoded at the *Gli-1* loci on the short arm of the first chromosome pair of each of the three coexisting so-called homoeologous genomes, A, B, and D, of bread wheat.

expressed in bread and durum wheats have already been described (6, 7). Their typical banding patterns are identified by lower case letters for each chromosome (8). Most of the ω -gliadins are encoded with γ -gliadins at *Gli-I* loci. They are identified in this article according to their chromosomal location as *Gli-A1*, *Gli-B1* and *Gli-D1* ω -gliadins. They can be characterized by their N-terminal sequence, their amino acid composition, their mass and their electrophoretic mobility (9–11). The names $\omega 1$ – $\omega 5$ refer to the acid-PAGE mobility of individual bands (9). The $\omega 5$ gliadins are part of the alleles encoded on chromosomes 1B, at the *Gli-B1* locus. They have been demonstrated to be major allergens in a specific form of food allergy called wheat dependant exercise-induced anaphylaxis (WDEIA; 12). The sequence of one allelic variant was recently published (13). It differs from ω -gliadins encoded at *Gli-A1*, and *Gli-D1* loci, not only by its N-terminal sequence, but also by the sequence of the repetitive, degenerated motif, whose repetition makes the large central repetitive domain characteristic of ω -gliadins. All the epitopes involved in WDEIA belong to this central domain (13–15). Some of these epitopes are also present on $\gamma 3$ -hordein, $\gamma 35$ - and $\gamma 75$ -secalins, which are, respectively, barley and rye prolamins (16). Recently, we showed that IgE from one patient with both contact urticaria and food allergy to gluten hydrolysates also cross reacted with $\gamma 3$ -hordein. Their pattern of reactivity with wheat, barley and rye proteins differed from that of a WDEIA patient tested in the same conditions. Inhibition experiments using the sera of these two

patients and a recombinant form of $\gamma 3$ -hordein, also suggested that differences existed between the epitopes involved in the allergic manifestations of these two patients (17).

The present work aims at evaluating first to which extent epitopes involved in immediate hypersensitivity to hydrolyzed wheat proteins (IHHWP) are different from those involved in WDEIA, by identifying the IgE reactive proteins of wheat at the allelic level and second at verifying if some alleles of $\omega 5$ -gliadins encoded at the *Gli-B1* locus, are also involved in IHHWP.

Materials and methods

Reagents were, unless specified, of analytical grade and from Sigma (St Louis, MO, USA). Immobilon-PTM, a PVDF membrane, was from Millipore Corp. (Bedford, MA, USA). Rabbit anti-human IgE-Horseradish peroxidase (HRP) conjugated antibodies, was from Dako S.A. (Trappes, France). SuperSignal West Dura Extended Duration[®] substrate for chemiluminescence detection was from Pierce Biotechnology (Rockford, IL, USA). Indian drawing ink was from Pelican (Hannover, Germany). Bread wheat grains (*Triticum aestivum* L.) were collected from bagged ears of 14 varieties grown at the INRA Plant Breeding Station in Clermont-Ferrand (France); they are listed in Table 1.

Patients

Four patients with IHHWP were selected among the six patients already described, which present a food allergy associated with contact urticaria to hydrolyzed wheat proteins (4). They all eat

Table 1. Identification and chromosome assignment of the alleles immunoreactive with the IgE from the four patients with immediate hypersensitivity to hydrolyzed wheat proteins

		Allelic gliadin patterns			IgE immunoreactive alleles identified			
Locus		<i>Gli-A1</i>	<i>Gli-B1</i>	<i>Gli-D1</i>	<i>Gli-A1</i>	<i>Gli-B1</i>	<i>Gli-D1</i>	Not assigned
Symbols used in Figs 1 and 2		★	●	◀	★	●	◀	
Wheat varieties	No.							
Chinese Spring	1	a(1)	a(3)	b(2)	k ₁ ; k ₂	a ₃	b ₁ ; b ₂	2
Soisson	2	k(3)	b(3)	b(2)		b ₃	b ₁ ; b ₂	2
Prinqual	3	f(1)	c(2)	b(2)		c ₂	b ₁ ; b ₂	5
Chopin	4	f(1)	d(4)	b(2)		d ₄	b ₁ ; b ₂	2
Arsenal	5	f(1)	e(3)	b(2)		e ₃	b ₁ ; b ₂	2
CampRémy	6	o(3)	f(3)	b(2)		f ₃	b ₁ ; b ₂	1
Feuvert	7	f(1)	g(4)	b(2)		g ₄	b ₁ ; b ₂	2
Rudi	8	o(3)	h(3)	b(2)		h ₂ ; h ₃	b ₁ ; b ₂	3
Insigna	9	f(1)	i(3)	i(2)		i ₃	i ₁ ; i ₂	3
Ruso	10	m(3)	m(2)	b(2)	m ₁ ; m ₂ ; m ₃	m ₃	b ₁ ; b ₂	2
Clément	11	k(3)	l(3)	b(2)	k ₁ ; k ₂	l ₁ ; l ₂ ; l ₃	b ₁ ; b ₂	2
Pandas	12	a(1)	m(2)	b(2)		m ₂	b ₁ ; b ₂	2
Goya	13	o(3)	q(2)	b(2)		q ₂	b ₁ ; b ₂	3
Salmone	14	a(1)	s(2)	b(2)		s ₂	b ₁ ; b ₂	5

The numbers used to identify each variety in this work are given in column 2. Corresponding multiallelic patterns for each chromosome are identified by letters in columns 3–5. Numbers in brackets indicate the number of bands presently identified in each pattern. The alleles immunoreactive with IgE are given in columns 6–9. They are marked in Figs 1 and 2 with either ★, •, or, ◀ for respectively *Gli-A1*, *Gli-B1* or *Gli-D1* alleles, when a chromosome assignment was possible. Subscripts columns 6–8 indicate the rank of the band by decreasing *Mr* in the SDS-PAGE pattern.

bread and pasta without any problem, but had strong allergic reactions when eating food containing HWP. Skin prick tests on these patients were positive, with HWP but negative with wheat flour and native proteins. Positive SPT result was defined as a wheal greater than or equal to one half of the diameter of the histamine control and at least 3 mm larger than the diameter of the glycerol-saline solvent used as negative control. Only patients with IgE reacting significantly on blot with ω -gliadin were selected. They correspond to patients 1, 2, 5 and 6 in the previous paper (4). Their IgE levels were low except for patient 1 (4). They were compared here to two other patients with characterized WDEIA triggered by unmodified wheat products. Two other control sera were purchased from PlasmaLab Int. (Everett, WA, USA), a normal human serum and the serum of an atopic patient allergic to peanut. The present study received the consent of the patients and the approval of the Ethical Committee of the Cochin hospital (Paris, France).

Electrophoretic analysis of grain proteins

Conditions were those already described (18). Briefly, gliadins were specifically extracted using 50% (v/v) propan-1-ol, directly from individual crushed grains of the selected 14 varieties. After evaporation of the solvent at 65°C, proteins were separated, using SDS-PAGE on a homogeneous gel with a total acrylamide plus bis-acrylamide content (T) of 10.3% and a bis-acrylamide content (C) of 1.3% (w/v).

Immunodetection of IgE reactivity

Gliadins, separated on SDS-PAGE, were blotted on PVDF membranes using the specific system of buffers described by Laurière (19). Proteins immunoreactive to serum IgE of patients were detected, using chemiluminescence as previously described (4). For an accurate identification of the immunoreactive gliadin alleles, the film with recorded chemiluminescence was superimposed on the PVDF membrane which was poststained for total proteins using Indian ink staining as already described (20). The Indian ink staining pattern was also compared with the protein pattern obtained from Coomassie Blue staining of a second control gel prepared in the same conditions as for the assay, in order to verify the efficiency of the transfer and to evaluate the protein amounts present in each band. No remaining proteins were detectable in the transferred gel.

Semi-quantitative analysis of protein-IgE reactivity

The chemiluminescence signals of each individual bands and the co-localized protein amount present in the original protein extracts were evaluated, respectively, using a densitometric analysis of the films with the recorded chemiluminescence and of the control gel, run in the same conditions but stained using Coomassie Blue. Scanning was performed using an Epson Expression 1680 Pro transparency unit (Seiko Epson corp., Japan). The Bio-1D software (Vilber Lourmat, Torcy, France) was used to assess the intensity of the chemiluminescent signal and of Coomassie blue staining. To allow comparisons between experiments, each band signal was expressed as a percentage of the total chemiluminescence recorded on the whole blot. The signal of individual band was also corrected for the corresponding protein quantity estimated from Coomassie blue staining. The sum of the resulting values for each wheat variety was used to compare the reactivity of the IgE of each patient with each variety.

Results

Electrophoretic patterns of gliadins

Typically three regions can be identified using SDS-PAGE. They correspond to α/β -, γ - and ω -gliadins, by decreasing mobility. The electrophoretic conditions used expend the ω -gliadin area and emphasize the polymorphism of the different alleles. The 14 varieties studied are listed in Table 1. They were chosen to display all the ω 5-gliadin alleles that were found at the *Gli-B1* locus in the 200 French and European cultivars, along with a control (no. 11) with 1BL/1RS translocation, depleted in ω 5-gliadins (18). These varieties show very different SDS-PAGE patterns (Fig. 1). Their patterns result from the simultaneous expression of the different allelic groups encoded on the three genomes. Each allelic group displays co-expressed associated typical bands, spread all over the different migration area of α/β -, γ - and ω -gliadins. The pattern of these bands is characteristic and allows the identification of each allelic group. Bands identified as a part of a multiallelic group were marked with the same lower case letter following the nomenclature of Jackson (8). In this paper, a subscript was added to the lower case letter to indicate the position of individual allelic bands in the SDS-PAGE pattern, according to their decreasing relative molecular mass (*Mr*). The banding patterns of the French varieties have been already determined (7, 18). The compositions of the varieties used in this work and the chromosome assignment of the expressed alleles are listed in Table 1, in columns 3–5. The

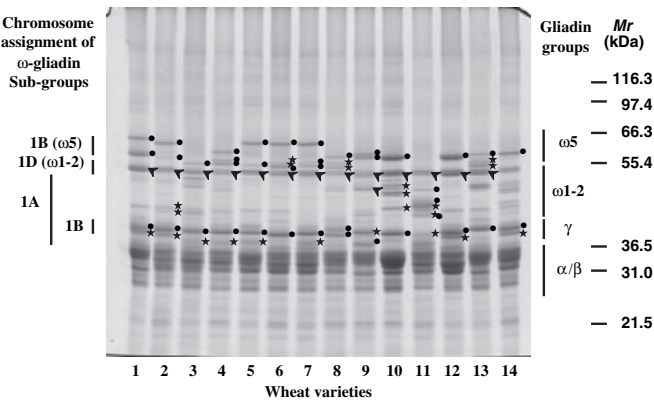


Figure 1. SDS-PAGE analysis of gliadins from each variety stained using Coomassie Blue. Lanes 1–14 refer to varieties listed in Table 1; 1B(ω 5) and 1D(ω 1–2) indicate the main positions in the pattern of the corresponding ω -gliadins, and the name according to Kasarda (9) into brackets; 1A, 1B, chromosome assignment and position of migration of bands always associated to ω -gliadins. Symbols ★, •, or ◀ on the figure, indicate on their left, co-expressed individual alleles, encoded respectively on genomes 1A, 1B or 1D. They are listed in Table 1, columns 3–5.

ω -gliadins of the 14 varieties, which are encoded at the *Gli-B1* locus, display a high polymorphism. On the contrary, genes of the *Gli-D1* locus encoding ω -gliadins in all varieties tested, express only two bands. In 13 varieties, they correspond to *Gli-D1b* alleles named here b_1 and b_2 . This pattern is present in 85.5% of the French varieties (18). The variety Insigna (no. 9) expresses the *Gli-D1i* alleles named here i_1 and i_2 . Concerning ω -gliadins encoded at the *Gli-A1* locus, only five multiallelic groups can be observed among the 14 varieties selected. As already shown (18), ω -gliadins encoded respectively on the three, *Gli-A1*, *Gli-B1*, *Gli-D1* loci, roughly migrate in distinct areas (see Fig. 1). For a convenient representation, the chromosomal origins of bands listed in Table 1, which reacted with IgE of patients with IHHWP or WDEIA, are marked with symbols in Fig. 1.

Detection of the IgE immuno-reactive gliadins

Four out of the six patients with IHHWP, already described with a food allergy associated with contact urticaria (3, 4), were shown to react significantly to ω -gliadins in preliminary blotting experiments. Concerning the two other patients, they correspond to patients 3, and 7 in the previous paper (4). The former patient displayed a pattern of IgE reactivity similar to that of the four patients used, but it was too fuzzy to be used for precise allele identification. The latter patient did not react significantly to gliadins. The sera of the four patients chosen were immuno-probed on blots with gliadins separated as in Fig. 1. The specificity of the reactions observed was assayed, using several controls. Each experiment was run along with a control blotted membrane immuno-probed without the patient serum. Two other control experiments were carried out. Membranes with wheat proteins fractions were immuno-probed with either a normal serum or the serum of an atopic patient allergic to peanut. Without patient serum or with a normal serum no reaction occurred with wheat proteins, in the conditions used. With the atopic serum, faint reactions were observed with the salt soluble proteins (results not shown), but no reaction occurred with gliadins (Fig. 2). In addition, two other patients' sera with WDEIA triggered by unmodified wheat products were used for comparison with IHHWP sera as exemplified in Fig. 3A and B. The specific IgE from each of the four IHHWP sera, displayed very similar patterns of reactivity, exemplified in Figs 2A, 3A and B respectively for patients no. 2, no. 1 and no. 5 (see reference 4). Almost the same bands were detected using the four sera. The main differences concerned the intensity of bands. These differences were not because of artifacts resulting from variations in the efficiency of blotting. Membranes were poststained with Indian ink to ensure a uniform presence of proteins on blots. Typically, and by

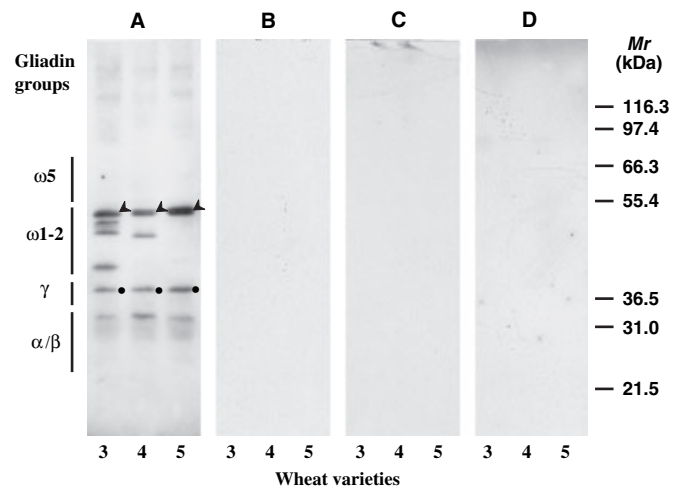


Figure 2. Specificity of the reactions observed using the sera of immediate hypersensitivity to hydrolyzed wheat proteins patients. Gliadins from wheat varieties no 3, 4, 5, separated using SDS-PAGE, as in Fig. 1. The same symbols ★, •, or ▲ are used for the identified alleles. Gliadins were immunoblotted with: (A) the serum from patient no. 2 (see reference 4) used as positive assay, and (B) without patient serum; (C), a normal serum from a nonallergic individual; (D) a serum from a patient allergic to peanut but without hypersensitivity to wheat, used as negative controls.

decreasing Mr , IgE reacted with undifferentiated b_1 and b_2 (or the homologous i_1 and i_2) alleles of ω -gliadins encoded at the *Gli-D1* locus, and with some alleles encoded at the *Gli-B1* locus. These last IgE reactive alleles had the lower Mr of the identified *Gli-B1* alleles. Except in the variety Clément (no. 11), most of them displayed one reactive band (two bands in Rudi, no. 8) in the γ -gliadin region. Because of its recombination with rye (1BL/1RS translocation) at *Gli-B1* locus, the variety Clément (no. 11) has no $\omega 5$ -gliadins, but it expresses in their place rye secalins. They correspond to *Gli-B1l* alleles which showed three reactive bands in the ω -gliadin area (Fig. 3). Additional bands from the *Gli-A1* locus corresponding to only the multialleles k and m, were also detected along with other not assigned bands, with various intensities depending on the patient. One of these last bands was common to all the varieties. It had the lower Mr of the reactive bands and migrated in the α/β region of the gliadin patterns. On the contrary, IgE from WDEIA sera reacted mainly with the *Gli-B1* alleles that displayed the higher Mr ($\omega 5$ -gliadins). Additional minor bands in the α/β - and γ -regions, were also detected in some varieties (results not shown). Only the reactivity of the two first varieties is presented for comparison, in Fig. 3. The reactivity of WDEIA sera with the 14 varieties are presented elsewhere (21). No overlapping was observed between the patterns of reactivity of WDEIA and IHHWP sera.

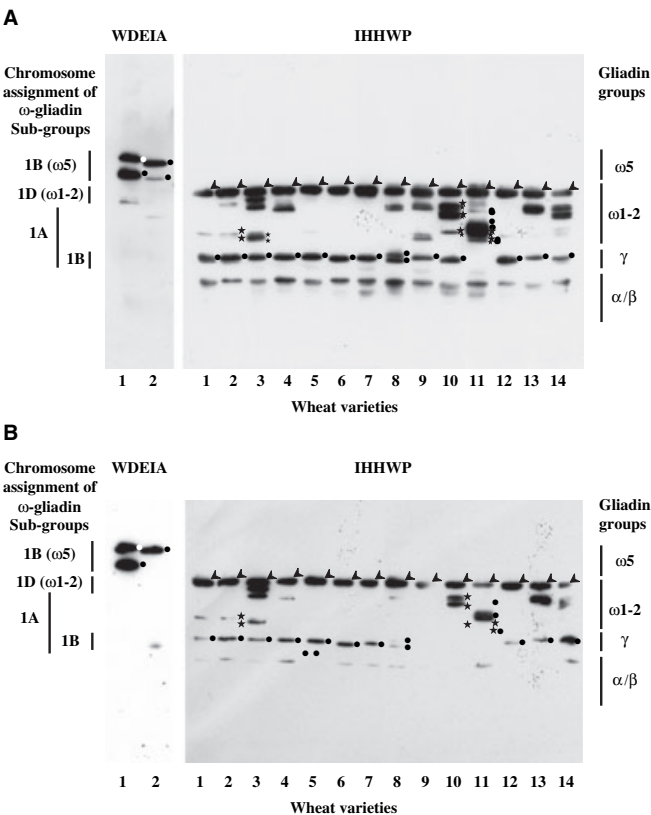


Figure 3. Gliadins immunoreactive with IgE of patients with immediate hypersensitivity to hydrolyzed wheat proteins. The same extracts as in Fig. 1, were used. Panels A and B, typical patterns of respectively patients no. 1 and no. 5 (see reference 4) taken as representative of the four patients. A comparison of the pattern obtained with wheat dependant exercise-induced food anaphylaxis patients with varieties 1 and 2 is shown on the left part. Symbols ★, ●, or ◀ on the figures, indicate on their left, the chromosome assignment of the individual identified alleles as in Fig. 1, which react with IgE. Their identity is listed in Table 1, columns 6–8. Unmarked bands could not be assigned.

Semi quantitative comparison of the reactivity of IHHWP sera with gliadins

Chemiluminescent light emission is directly proportional to the amount of peroxidase conjugates fixed on the blot, allowing thus a quantitative evaluation of the IgE fixed on their antigens. Films with the recorded chemiluminescence were scanned to measure this signal and to evaluate the relative IgE reactivity of each band within each experiment. The objective was to compare the overall reactivity of the different varieties for each patient, in order to detect if some varieties were less IgE reactive. Results are presented in Fig. 4. The differences of IgE reactivity observed concerned both the varieties and the patients. The serum of patient 6 (fourth bar in Fig. 4) reacted more strongly with the varieties 1–5 than the

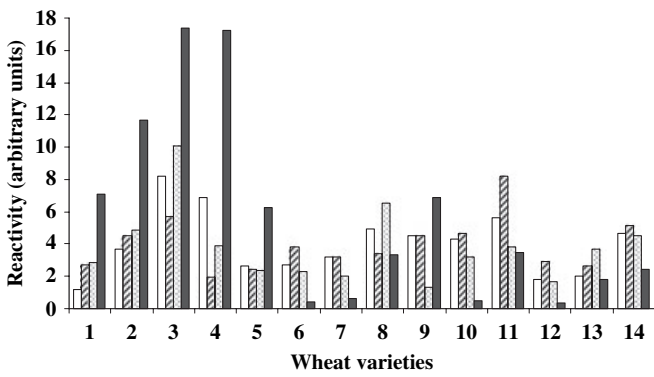


Figure 4. IgE reactivity of IHHWP patients. Semi quantitative evaluation of the total IgE reactivity with gliadins from each wheat variety, with sera from patients with immediate hypersensitivity to hydrolyzed wheat proteins. Individual bars represent the cumulative chemiluminescence band signals obtained from the serum of each of the four patients, with each of the 14 varieties, listed on abscises. Patients number 1, 2, 5, 6 (four successive bars) and calculations are described in (4) and in *Material and methods*.

other sera. Concerning the varieties, wheat variety no. 3 was the more reactive, and the varieties no. 6, 7, 12 and 13, were the less reactive with the four sera.

Discussion

The four IHHWP patients with associated food allergy displayed similar clinical symptoms to those of WDEIA patients, following wheat food intake. Both types of patients displayed generalized urticaria or anaphylaxis and they generally involved anti- ω -gliadin IgE. The main differences were the negativity of SPT with normal unmodified wheat products, their positivity with HWP and the apparent absence of associated effort, in the cases of IHHWP patients. This study is a further attempt to discriminate both diseases on the basis of the specificity of the IgE they involved. Recently (17) we showed that an IHHWP patient reacted like WDEIA patients with $\gamma 3$ -hordein, a barley prolamin cross-reacting with $\omega 5$ -gliadins (16). For this reason, bread wheat varieties used to characterize the IgE were chosen to be representative of the $\omega 5$ -gliadin alleles expressed in French bread wheat varieties and to which the French population has been exposed during the past 20 years. The objective was to screen the different alleles of ω -gliadins and more particularly of $\omega 5$ -gliadins, for the extent of their implication in IHHWP. Among the selected varieties, Clément (no. 11), was introduced as representative of wheat varieties that present 1BL/1RS translocation and so are deprived of $\omega 5$ -gliadins. These last varieties express in their place secalins (*Gli-B1* alleles), which are similar to ω -gliadins. Omega gliadins of the three genomes expressed by all

the selected varieties, along with their corresponding encoding loci on wheat chromosomes, could be distinguished with accuracy, according to both their relative mobility and to the characteristic band pattern they displayed upon SDS-PAGE. These patterns were the result of constitutive co-expression of gliadins inherited all together, in the grains. The reproducibility of these patterns is a basis of wheat variety identification. The combination of these gliadin properties with an accurate protein transfer and the possibility to localize accurately the chemiluminescent signal on films by superimposition on the membranes poststained for total proteins, allowed a nonambiguous identification of the IgE reactive alleles. The results showed that none of the IgE of the four patients tested sensitized to HWP reacted with ω 5-gliadins. The reactive ω -gliadins were encoded mainly at *Gli-D1* and *Gli-B1* loci. Most of the alleles of ω -gliadins and γ -gliadins encoded at *Gli-A1* did not react. Only the alleles k1, k2 and m1, m2, m3, expressed in the varieties Soisson (no. 2), Ruso (no. 10) and Clément (no. 11), were reactive with IgE. This shows the singularity of these alleles among the others which are also encoded on chromosome 1A. Concerning the *Gli-B1* alleles reacting to IHHWP sera, they reacted in most cases as a single band in the γ -gliadin area. This result is in accordance with our previous observation that the serum from a patient with IHHWP, also reacted with γ 3-hordein, which was homologous to wheat γ -gliadins (17). The *Gli-B1* locus is known to encode both γ - and ω -gliadins. It is not known if these γ -gliadins share common epitopes with the reacting ω -gliadins encoded by *Gli-D1*, or if they are different antigens reacting with different IgE clones. It is interesting to remind here that in addition to barley hordeins, IgE from IHHWP patients also reacts with secalins from rye (17) (and additional results not shown). This is confirmed here by the reactions of IgE from only IHHWP patients with the *Gli-B1* alleles, which are rye secalins and which replace ω 5-gliadins, in the variety Clément (no. 11). This suggests that secalins structurally related to ω 1,2-gliadins or to γ -gliadins encoded respectively by *Gli-D1* and *Gli-B1*, also exist in rye. As IgE from WDEIA patients did not react to these *Gli-B1* alleles (21), these homologies must be distinguished from those between ω 5-gliadins, γ 3-hordeins, γ 35- and γ 75-secalins which were already revealed by using IgE from WDEIA patients (16, 17, 21). These observations show that wheat, rye and barley could be potentially harmful not only to WDEIA patients but also to IHHWP patients. These plants are phylogenetically related, and the immunological cross-reactions are normal. Prolamins from these cereals share general common structural features, and strong immunological homologies of these proteins, within species and between species, which are different from those cited above, are known since a long time. This makes highly difficult to raise specific antibodies reacting with specific

components of prolamins. Compared with the IgG raised experimentally, IgE from both IHHWP and WDEIA patients exhibited exceptional high specificities. They were able to discriminate between strongly related gliadins encoded on homoeologous chromosomes, like ω 5- and ω 1,2-gliadins. This shows that the epitopes against which IgE antibodies are elicited in IHHWP and WDEIA, are probably different from the common epitopes which are tolerated in normal individuals eating wheat products (22, 23). The involvement of these IgE epitopes is probably the result from several factors. Among them, modifications of the structure of the antigens, exposing buried structures, or modifications in their presentation to the immune system other than by the oral route, are possible factors. In the present case of IHHWP, both these factors occurred. The wheat protein antigens have been broken by partial hydrolysis, leading to the exposition of internal structures and to random aggregation of the fragments, as already observed (4). These modifications, combined with the application on the skin of these new antigens, more than their oral consumption, were probably determinative in the orientation of the immune system to an IgE response. Allergic sensitizations following epicutaneous exposure to proteins are now recognized (24, 25).

Conclusion

The antigens involved in IHHWP associated with food allergy and in WDEIA are very similar but they are different. They correspond respectively to specific ω 1–2 and to ω 5-gliadins. No IgE cross reactions were observed between these antigen groups. Thus IgE elicited in these two diseases distinguish between the gliadin variants encoded either at the same loci, or on homoeologous loci on the three genomes of bread wheat. This shows that WDEIA and a form of IHHWP, which are both food allergies triggered by wheat proteins, differ not only by symptoms but also by the specificity of the IgE elicited against gliadins in each pathology.

Individual susceptibilities of patients can explain these differences. However, the modifications of the structure of the proteins induced by industrial processes to make new ingredients, combined with new end uses of wheat proteins involving skin application, can orient the immune system leading to specific pathological reactions.

The high specificity of the IgE involved in IHHWP and WDEIA, which discriminate not only between alleles among ω -gliadins, but also among other gliadin groups, could be a potential way to discriminate these two allergies and to establish which type of ingredient the patient must avoid in food or cosmetics.

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