

WHEAT FLOUR ALLERGY: AN ENTIRE DIAGNOSTIC TOOL FOR COMPLEX ALLERGY

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SUMMARY: Wheat proteins are involved in respiratory allergy, contact allergy and food allergy. Wheat allergens involve in these pathologies are well-known. However, establishment of wheat allergy diagnostic can be sometimes difficult on account of the complex allergenic composition of skin prick test (SPT) solutions of wheat flour. Therefore, we have studied specific IgE reactivity from patient sera with wheat food allergy, and characterized allergenic composition of wheat SPT solutions by specific antibodies directed to wheat allergens.

The results showed that 20 of the 25 sera analyzed contained specific IgE to at least one wheat protein fraction. Among positive sera, 75% have specific IgE to water/salt soluble fraction, 85% to native gluten fractions and 65% to wheat isolate fraction. The results showed also that SPT solutions of wheat flour contained major food allergens from each allergenic fraction.

These results highlighted the importance of using fractions, which constitute the whole wheat allergenic pattern, during specific IgE reactivity analyses. Moreover, we have observed that wheat isolate extract (results of food industrial process) contained not only modified allergens (neo-allergens) involve of specific food allergy to wheat isolate but also some native allergens involve in wheat food allergy. Thus, these results showed the importance to use, for wheat *in vivo* diagnosis together wheat SPT solutions (gluten extract and wheat isolate) in order to differentiate wheat food allergy to specific wheat isolate allergy.

Key-words: Wheat flour allergy - Specific IgE antibodies - Allergenic extracts - ω_5 -gliadins.

INTRODUCTION

Wheat is the most widely cultivated cereal and an important source of food and dietary protein worldwide. Wheat proteins are involved in the three routes of sensitization: inhalation, contact and ingestion. In these three allergies, wheat allergens are present in various fractions of wheat flour. Wheat flour is an important cause of baker's asthma, a well-known occupational respiratory allergy to inhaled flour. The major allergens (α -amylase/trypsin inhibitor family) in this allergy are albumin proteins, which are present in the water/salt soluble fraction (1, 2). For contact allergy, wheat hydrolyzed proteins can be involved of contact sensitization (3). These gluten hydrolysed proteins can be found generally in cosmetic products. In addition, wheat food allergens are found in all wheat flour protein fractions (water/salt soluble (albumins and globulins), native gluten (gliadins and glutenins) and wheat isolate fraction) (2). Recently, neo-allergens in wheat isolate extracts (used in the food industry) have been involved in food allergy (4, 5). These patients could also eat cereal-based products without any problem.

Nevertheless, extraction of all wheat allergens requires several steps an account of the insolubility of the majority of wheat flour proteins in standard water/salt solutions. Thus, we studied sera from patients with accurate diagnostic of wheat food allergy. The reactivity of specific IgE antibodies was evaluated for various wheat protein fractions and wheat isolate. Moreover, the allergenic composition of skin prick test solution (SPT) of wheat flour was analyzed with specific antibodies directed to wheat allergens.

METHODS

Sera were obtained from 25 patients with wheat food allergy, suffering from various clinical symptoms (atopic dermatitis, asthma, eczema, urticaria, gastrointestinal symptoms, systemic anaphylaxis and wheat-dependent exercise-induced anaphylaxis (WDEIA)). Food allergy to wheat was established by skin prick tests and by standardized oral challenge. The reactivity of specific IgE was studied by enzyme-linked immunosorbent assay (ELISA) to: native wheat fractions (water/salt soluble fraction (albumins/globulins), ethanol-soluble fraction (gliadins), insoluble gluten fraction (glutenins)), wheat isolate fraction and SPT solutions of wheat flour. The binding IgE specificity was analyzed by immunoblotting after SDS-PAGE electrophoresis of water/salt soluble and gliadin fractions.

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Also, antibodies specific for total gliadin fraction, ω_5 -gliadins, low and high molecular weight (LMW and HMW) glutenin subunits or lipid transfer protein (LTP) (6, 7) were used by ELISA to analyse the allergenic composition of wheat SPT solutions.

RESULTS

The reactivity of specific IgE antibodies from 25 sera of patients for native and modified wheat allergenic fractions was determined by ELISA. Our results showed

Sera	Water/salt soluble fraction	Native gluten		Wheat isolate fraction
		Gliadins	Glutenins	
1	0	3	0	5
2	6	6	4	6
3	2	2	3	2
4	3	4	4	4
5	3	3	nd	3
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	3	3	nd	3
10	0	0	2	0
11	2	0	0	0
12	3	2	nd	2
13	3	3	2	0
14	0	0	2	0
15	0	0	0	0
16	4	3	nd	4
17	0	3	2	2
18	2	2	nd	0
19	0	0	2	4
20	0	0	0	0
21	3	0	nd	0
22	2	2	nd	3
23	3	0	nd	0
24	3	3	nd	3
25	2	2	3	3

Table 1: Specific IgE class for different wheat protein fractions (native or modified) obtained in ELISA. nd : not determined.

that 20 sera contained specific IgE to at least one wheat protein fraction (table 1). Among positive sera, 75% (15) have specific IgE antibodies to water/salt soluble fraction, 85% (17) to native gluten fractions (gliadins and glutenins) and 65% (13) to wheat isolate fraction.

Immunoblotting analyses were performed with water/salt soluble and gliadin fractions. Among the 25 analyzed sera, results showed that 64% (16) of patients have IgE antibodies to proteins of wheat fractions. Among these 16 positive sera, 63% (10) of patients have IgE antibodies to water/salt soluble fraction and 94% (15) to gliadin fraction. Moreover, sera of WDEIA patients have specific IgE binding to ω_5 -gliadin allergens of gliadin fraction (figure 1).

Qualitative analyses of allergenic content of wheat SPT solutions were performed by ELISA with polyclonal antibodies specific for different wheat flour allergens: total gliadin fraction, ω_5 -gliadins, low and high molecular weight (LMW and HMW) glutenin subunits and lipid transfer protein (LTP) and with specific IgE

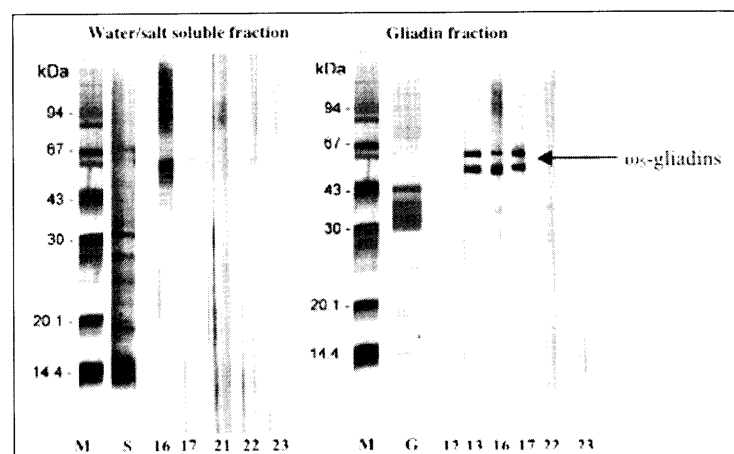


Figure 1: IgE specificity of 7 sera for water/salt soluble and gliadin fractions by immunoblotting after separation by SDS-PAGE.

M: Molecular weight markers

S: Water/salt soluble proteins separated by SDS-PAGE and visualized by silver staining coloration.

G: Gliadin proteins separated by SDS-PAGE and visualized by silver staining coloration.

12 to 23: Specific IgE binding of 7 sera of patients with wheat food allergy.

Specific IgE and antibodies detections to:		SPT allergenic solutions of wheat flour		
		Wheat soluble extract	Gluten extract	Wheat isolate extract
Water/salt soluble fraction	Albumins (LTP, ...)	+++	-	-
	Globulins	+++	-	-
Native gluten fraction	Gliadins (ω_5 -gliadins, ...)	-	+++	++
	Glutenins	-	+++	++
Modified gluten fraction	Neo-allergens	-	-	+++

Table 2: Detection of allergens by specific IgE antibodies and wheat specific antibodies in wheat SPT solutions determined by ELISA.

antibodies from patients with food allergy. Results showed that SPT solutions of wheat flour contained major food allergens from each allergenic fraction (table 2). Wheat soluble extract contained albumins and globulins allergens as LTP, gluten extract contained native gliadins and glutenins and wheat isolate contained modified allergens, specifically recognized by some patients (neo-allergens). Moreover, wheat isolate contained also native allergens which were not modified by food industrial process.

DISCUSSION

In this study, we have investigated the reactivity of specific IgE antibodies from patients with food allergy to various wheat protein fractions and SPT solutions of wheat flour. Moreover, we have analyzed allergenic composition of wheat SPT solutions with specific antibodies to various wheat food allergens (LTP, ω_5 -gliadins,...). Our results were in good agreement with those from other studies on wheat food allergy regarding IgE reactivity of wheat proteins (1, 2, 8). These results highlighted the importance of using different fractions, which constitute the whole wheat allergenic pattern, during specific IgE reactivity analyses. We can also note that all sera of patients with WDEIA contained specific IgE to ω_5 -gliadins. According to previous studies, these results confirmed that ω_5 -gliadins were major allergens from patients with wheat-dependent exercise-induced anaphylaxis (8, 9).

The results obtained with wheat specific antibodies shown that wheat SPT solutions contained the principal food allergens in each fraction: albumins/globulins and LTP in wheat soluble extract and gliadins, glutenins and ω_5 -gliadins in gluten extract. Moreover, we have observed that wheat isolate fraction (results of food industrial process) contained not only modified allergens (neo-allergens) involve in specific food allergy to wheat isolate (4, 5) but also some native allergens involve in wheat food allergy. Thus, these results showed the importance to use for wheat in

vivo diagnosis some wheat SPT solutions (gluten extract and wheat isolate) in order to differentiate wheat food allergy to specific wheat isolate allergy.

These data show one more time, the importance of using complete tools, which take into account of various specificities of wheat flour allergenic extracts to improve the diagnosis sensitivity of the wheat food allergy. Finally, this study highlights also the particularity of certain allergens as ω_5 -gliadins in wheat allergy and the benefit of a new tool containing this purified allergen to make easier the WDEIA diagnosis.

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