

The maize major allergen, which is responsible for food-induced allergic reactions, is a lipid transfer protein

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Background: Cereals are the most important nutritional component in the human diet. Food-induced allergic reactions to these substances therefore have serious implications, and exhaustive diagnosis is required. Such diagnosis is still difficult because of the incomplete knowledge about major cereal allergens. In particular, few food-induced allergic reactions to maize have been reported, and no information on the allergenic proteins is available.

Objective: Having observed several anaphylactic reactions to maize, we planned a study to identify maize major allergens and cross-reactivity with other cereals, as well as to peach because the majority of patients also reacted to Prunoideae fruits.

Methods: Twenty-two patients with systemic symptoms after maize ingestion and positive skin prick test responses and serum-specific IgE antibodies to maize were selected. The IgE-reactivity pattern was identified by SDS-PAGE and immunoblotting. The major allergen identified was then purified by HPLC and characterized by mass spectrometry, determination of the isoelectric point value, and N-terminal amino acid sequencing.

Results: Sera from 19 (86%) of the 22 patients recognized a 9-kd protein, thus confirming this as the maize major allergen. This protein had an isoelectric point of greater than 9, a molecular mass of 9047.0 d, and no glycosylation. Determination of its N-terminal sequence showed that it was a lipid transfer protein (LTP). By using immunoblotting-inhibition experiments, we demonstrated that the LTP cross-reacts completely with rice and peach LTPs but not with wheat or barley LTPs. N-terminal sequence of the 16-kd allergen (recognized by 36%

of patients) showed it to be the maize inhibitor of trypsin. This protein cross-reacts completely with grass, wheat, barley, and rice trypsin inhibitors.

Conclusion: The major allergen of maize is an LTP with a molecular weight of 9 kd that is highly homologous with the peach LTP, the major allergen of the Prunoideae subfamily. (*J Allergy Clin Immunol* 2000;106:744-51.)

Key words: Maize allergens, lipid transfer protein, food allergy

Cereal grains belong to the Gramineae botanical family, which is a well-known cause of allergic and immunologic reactions. Cereals include important crops, such as wheat, barley, rye, oats, rice, and maize, and are thus the major components of the human diet throughout the world. The implications of sensitization to any of these are consequently of major consequence. The different consumption patterns of the various cereals around the world may justify the different rates of sensitization to each cereal. For example, wheat is more frequently reported as a cause of food allergy in the United States, as demonstrated by a double-blind, placebo-controlled, food challenge (DBPCFC) for 6 cereals in a study by Jones et al,¹ whereas rice is the chief cause of allergy in Asiatic countries.² However, there are other factors capable of increasing the risk of IgE-mediated reactions to each cereal. One such factor may be cross-reactivity with other food allergenic sources frequently consumed in a given country. In the Mediterranean area peach is one of the most frequent causes of food-induced allergic reactions, as demonstrated in Italy, Israel, and Spain.³⁻⁵ We recently identified a 9-kd lipid transfer protein (LTP) as the major allergen of peach⁶ and were very surprised to find a high homology in its amino acid sequence with LTPs of vegetable foods of very different botanical origins, such as maize. The literature provides few cases of food-induced allergic reactions to maize. Recently, an episode of exercise-induced anaphylaxis caused by maize was reported by Pauls and Cross.⁷ Eight patients had positive DBPCFC results for maize in the study by Jones et al¹ mentioned above, and a few cases in children have been reported by Bock et al.⁸ The protein composition of maize seeds has been investigated in depth, but few studies have addressed the identification of maize

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Abbreviations used

DBPCFC: Double-blind, placebo-controlled, food challenge
LTP: Lipid transfer protein
MW: Molecular weight
OAS: Oral allergy syndrome
PVDF: Polyvinylidene difluoride

allergens. The only known maize-seed allergen is an amylase-trypsin inhibitor⁹ that is homologous to other members of this family in other cereals and is responsible for the so-called baker's asthma. The literature reports that the major allergen of maize is a protein belonging to group 1 grass pollen allergens that is responsible for seasonal respiratory symptoms. Cross-reactivity with grass pollen is the most common cause of false-positive RAST results to cereal grains¹ in patients without food-induced allergic reactions.

The aim of this study was to identify the major allergens of maize seed responsible for food-induced allergic reactions, as well as to characterize these allergens by evaluating both their immunochemical properties and their allergenic cross-reactivities with other cereals and with grass pollen and peach fruit.

METHODS

Patients

Patients referred to the Allergy Centre of the 3rd Department of General Medicine, University of Milan, or to the Bizzozzero Division of "Niguarda Ca' Granda" Hospital, Milan, were selected on the basis of documented food-induced allergic reactions to maize. All patients who reported systemic anaphylaxis after eating maize in different forms (eg, polenta, popcorn, or crisps) were recruited for the study. A detailed clinical history revealed that before presenting systemic symptoms, some patients had experienced oral allergy syndrome (OAS) to maize. The history of allergic reactions to other cereal crops, fruits, or vegetables and to other foods was carefully evaluated in each patient. Because of the severity of the symptoms, only IgE serum detection for maize was evaluated; in vivo tests, skin tests, and provocation tests were avoided.

Skin prick tests with commercial food extracts (1:10 wt/wt) and with fresh fruits and vegetables with the prick-prick¹⁰ technique were performed for a selection of foods. The history of OAS for fresh fruit was verified by open challenge with a routine method.¹¹ The presence of pollinosis was evaluated on the basis of the history of respiratory symptoms during the pollen season and skin prick test positivity for commercial pollen extracts (Bayer-DHS, Milan, Italy). The extracts used were Alphatest, standardized in activity units, for birch and hazel pollens, grasses, *Parietaria* species, mugwort, and ragweed.

Sera, stored at -70°C, from all the patients were used to perform the in vitro part of the study.

In vitro methods: Identification of maize major allergens

Allergen extract. Maize proteins were extracted by using a commercial flour diluted 1:5 in ammonium hydrogen carbonate buffer (0.125 mol/L, pH 7.8, [NH₄]HCO₃) and stirred overnight at room temperature.¹² The sample was centrifuged at 18,000 rpm for 50 minutes at 4°C, filtered, and desalted by using a Hi Trap Desalting

column (Pharmacia Biotech, Uppsala, Sweden), according to the manufacturer's instructions.

The protein concentration of maize flour, determined by using the Bradford¹³ assay, was 0.47 mg/mL.

Maize extract was concentrated twice with disposable microconcentrators (Centricon 3, cut-off value of 3000 d; Amicon, Inc, Beverly Millipore Corp, Bedford, Mass), obtaining a protein concentration of 0.99 mg/mL, as determined by using a Bradford assay.

SDS-PAGE immunoblotting. Proteins were separated in a discontinuous buffer system with an SDS-PAGE gel (6% stacking gel and a 7.5%-20% separation gradient gel) essentially as described by Neville.¹⁴ Maize extract diluted 1:2 in sample buffer (4 mg/cm of gel) was run in a BIO-RAD Protein Ilxi vertical electrophoresis slab cell (BIO-RAD Labs, Richmond, Va).³

After separation, the part of the gel containing the molecular weight (MW) markers and maize extract was stained with Coomassie brilliant blue R-250 (Pharmacia), and the other part of the gel was electroblotted either onto a nitrocellulose membrane (0.45 µm; Amersham, Buckinghamshire, UK) or onto a polyvinylidene difluoride (PVDF) hydrophobic membrane (ProBlott, Applied Biosystem, Foster City, Calif) by using a trans-blot cell from BIO-RAD at 0.45 A and 100 V for 4 hours at 4°C.¹⁵ The unoccupied protein-binding sites were blocked by incubation with PBS (pH 7.4) with 0.5% Tween-20. The nitrocellulose membrane was then cut into strips, which were incubated overnight with each patient's serum and a negative serum as a control. IgE binding was detected by incubation with iodine 125-labeled anti-human IgE diluted 1:4 in blocking solution and exposed on autoradiographic film (Hyperfilm, Amersham) in exposure cassettes at -70°C for 4 days. The immunoblotting results indicated that the major allergen of maize was a protein with a MW of approximately 9 kd. All the purification procedures were thus carried out on this 9-kd protein. Its subsequent identification as an LTP with a high amino acid sequence homology with peach LTP confirmed the importance of studying its cross-reactivity not only with other cereals but also with peach fruit, as we had hypothesized.

To determine the N-terminal sequences of the allergenic proteins, the electroblotted PVDF membrane was analyzed on an Applied Biosystems 470A gas-phase sequencer (Applied Biosystem, Foster City, Calif).

In vitro methods: Characterization of the 9-kd allergen

Purification by HPLC. The 9-kd protein from maize was purified by cationic exchange chromatography with an HPLC System (AKTA Purifier; Pharmacia Biotech, Uppsala, Sweden) on a Resource-S (6 mL) column (Pharmacia Biotech). The column was eluted with a salt gradient from 0 to 1 mol/L NaCl in 0.03 mol/L sodium citrate dihydrate buffer (pH 6). As the SDS-PAGE analysis of the collected fraction showed, the 9-kd component was not pure, and a second purification step by using gel filtration was required.

Cationic exchange-concentrated fractions were separated on a Superdex 75 column equilibrated and eluted with 0.15 mol/L NaCl in 0.03 mol/L sodium citrate buffer (pH 6). A calibration run was performed with standard proteins at known MWs (Gel Filtration Calibration Kit, Pharmacia Biotech).

The maize sample injection volume was 450 µL, and the chromatogram was monitored at 280 nm.

Assessing the purity of the 9-kd protein and IgE-binding capacity. The purity of the 9-kd protein was assessed by using SDS-PAGE immunoblotting with the purified protein, according to the method described above.

Amino acid composition and isoelectric point value of the 9-kd protein. The 9-kd protein was characterized by amino acid sequencing and isoelectrofocusing, as described elsewhere.⁶

Mass spectrometry analysis. The mass spectrum was produced by a Finnigan MAT ThermoQuest (San Jose, Calif) LCQ ion trap mass spectrometer by using a nanospray source (Protana, Odense, Denmark). Ten microliters of the purified maize LTP after dialysis against water was diluted with 10 μ L of the methanol containing 1% formic acid. Two microliters of the mixture was applied to the nanospray source, and data were acquired for several minutes. Bio-mass software was used for the deconvolution calculation.

In vitro methods: Cross-reactivity with other cereals, peach, and grass pollen

Cross-reactivities between maize and wheat; barley, rice, and grass pollen; and maize and peach extract were evaluated by immunoblotting-inhibition experiments.

Allergen extracts. Grains of wheat, barley, and rice were milled to obtain flour, and proteins were extracted by using the methods described elsewhere.¹² Wheat extract was lyophilized at a concentration of 40 mg (wt/vol), and all samples were stored at -20°C .⁸ Timothy grass pollen was supplied by Pharmacia and Upjohn. Peach extract was prepared by using fresh peel, according to the method of Bjorksten et al.¹⁶

The protein concentrations of barley, rice, wheat, peach, and grass pollen were determined by the Bradford method as being 1.80 mg/mL, 2.34 mg/mL, 2.32 mg/mL, 4.2 mg/mL, and 13.3 mg/mL, respectively.

Maize blot inhibition by grass pollen and flour extracts. Briefly, samples of 500 μ L of pooled sera from all the patients studied were inhibited with 500 μ L of grass, barley, wheat, and rice extracts either undiluted or at different dilutions. The protein content of each inhibitor was as follows: grass pollen of 1.5, 0.15, 0.015, and 0.0015 mg; barley of 1.5, 0.15, 0.015, and 0.0015 mg; wheat of 5 and 0.5 mg; and rice of 1.35 and 0.135 mg. After preincubation, the immunoblotting of maize was performed as described above.

Blotting cross-inhibition between maize and peach extract. We performed an immunoblotting of peach extract (0.3 mg/cm of gel) inhibited by 3, 0.3, or 0.03 mg of maize extract and an immunoblotting of maize inhibited by 4.83, 0.483, or 0.0483 mg of peach extract. Maize and peach immunoblotting was performed simultaneously with the same pooled sera and according to the steps described above.

RESULTS

Patients

Twenty-two patients, 16 women and 6 men, aged from 12 to 50 years (mean age, 28.4 years) were recruited for the in vitro study. All had severe systemic reactions to maize documented by visits to hospital emergency departments. Three had exercise-induced food-dependent anaphylaxis. Nine patients reported OAS before experiencing systemic symptoms. The demographic data, maize-specific IgE values (CAP System), symptoms elicited by maize, pollens responsible for respiratory symptoms, and history of reactions to other foods, including other cereals, are reported in Table I. Ten patients presented with OAS to peach, of whom 5 had systemic symptoms. Some patients were sensitive to other members of the Prunoideae subfamily. Four patients had histories of severe anaphylactic reactions to nut group members (almond, hazelnut, and walnut), 6 to rice, 1 to spelt, and 3 to barley derivatives.

Major allergens of maize

The immunoblots showed that the sera of 19 (86%) of 22 patients reacted with a 9-kd band, that the sera of 8

(36%) of 22 recognized a 16-kd protein, and that only the sera of 5 (22.7%) of 22 reacted with higher MW bands (range 25-85 kd; Fig 1). Thus the 9-kd protein is the major allergen of maize responsible for food-induced allergic reactions.

N-terminal sequence

The 9-kd major allergen and the 16-kd protein were characterized by analyzing the N-terminal sequence of the allergenic proteins blotted on PVDF membranes. The results showed the following N-terminal sequence of the 9-kd protein: Ala-Ile-Ser-Cys-Gly-Gln-Val-Ala-Ser-Ala-Ile-Ala-Pro-Cys-Ile. Research conducted with a database identified this protein as maize LTP, the 120-amino acid sequence of which was described in 1988. The amino acid sequence of our protein corresponded to amino acids 28 to 42 of the published sequence and revealed a very high degree of identity (Swiss Prot Data Bank, accession number P19656) with several proteins belonging to the LTP family, especially rice (79%), peach (63%), and apricot (60%) LTPs.

The N-terminal sequence of the 16-kd protein was as follows: Ser-Ala-Gly-Thr-Ser-Cys-Val-Pro-Gly-Trp-Ala-Ile-Pro-His-Asn-Pro. This protein was identified as the maize inhibitor of trypsin/activated Hageman factor (XIIA) through a search in the Swiss Prot Data Bank (accession number P01088).

Purification of the maize 9-kd allergen by HPLC

Fig 2, A, shows the chromatographic profile with only two peaks, as obtained from the cationic exchange column. SDS-PAGE analysis of the second fraction revealed the presence of the 9-kd component and of other higher MW proteins. Several amounts of the impure fraction were collected and concentrated to a final volume of 450 μ L with a protein concentration of 0.41 mg/mL. This fraction was loaded onto a gel-filtration column, obtaining the 4 peaks in Fig 2, B. The third peak, corresponding to the purified 9-kd protein, was collected, concentrated to a final volume of 650 μ L with a protein content of 36 μ g/mL, and analyzed by SDS-PAGE.

Purity of the 9-kd protein and its IgE-binding capacity

The 9-kd protein, analyzed either by SDS-PAGE or by immunoblotting with the pooled sera, was pure. The IgE-binding capacity of the fraction was demonstrated in this experiment (Fig 2, right).

Isoelectrofocusing

Isoelectrofocusing gave an isoelectric point value of greater than 9 for the protein at 9 kd.

Mass spectrometry

The MW of the purified maize LTP calculated by deconvolution software was 9047.0 d, corresponding to the MW deduced from the already known sequence; this excludes the presence of glycoforms.

TABLE I. Patient characteristics

Patients	Sex	Age (y)	Symptoms	Maize CAP system (kUA/L)	Pollens	Other cereals causing symptoms	Other foods causing symptoms
1	F	20	OAS, GE	4.76	Grass, ragweed, <i>Parietaria</i> species, mugwort, birch, hazel		
2	F	21	OAS, GE	4.34	Grass		
3	F	12	Urticaria, GE	0.438	Grass, birch, hazel, mugwort		
4	M	50	OAS, GE, urticaria	2.28	Grass		Peach
5	F	24	OAS, hypotension	8.70	Grass		Peach, apricot
6	F	28	Angioedema, GI	18.5	Grass, mugwort	Wheat	Peach
7	F	44	OAS, urticaria, GE, dyspnea	1.51	Grass, mugwort		Apricot
8	F	31	Shock	6.76			Peach, pea, pear, cherry, broadbean, apricot, celery, walnut, hazelnut, peanut, kiwi, lettuce
9	F	18	Shock	19.4	Grass	Rice, barley, oats	Peach, apricot, plum, cherry, tomato, walnut, hazelnut, almond, melon, apple, celery, onion, sweet pepper, asparagus, soybean
10	M	19	OAS, GE	60.9	Mugwort		Peach, apricot
11	M	41	Asthma	7.21		Rice, wheat, barley	
12	F	16	Dyspnea, GE	11.5	Grass, ragweed		Peach, cherry, plum, apricot
13	F	28	OAS, urticaria	13.7	Birch, hazel	Rice	
14	F	29	EFA	1.20			Peach, cherry, plum, apple, apricot, onion, garlic, pear, celery, hazelnut, walnut
15	M	30	OAS GE	1.12	Grass, birch, mugwort, ragweed		Peach
16	F	25	OAS, GE, GI	2.71	Grass	Barley	Grape, fennel, walnut
17	M	27	EFA	2.10			Peach, apricot, cherry, walnut
18	F	25	Shock	1.80		Rice, spelt, barley, oats	Peach, plum, cherry, apricot
19	M	30	Dyspnea, urticaria	15.8		Rice, barley	
20	F	30	Shock	2.04	Grass		Cherry, apricot, peach, plum
21	F	44	Urticaria, GE	4.27		Rice	Kiwi
22	F	34	EFA	0.362	Grass	Rice	Peach, cherry, plum, apricot

GE, Glottis edema; GI, gastrointestinal; EFA, exercise-induced food-dependent anaphylaxis.

Inhibition of maize immunoblotting

Inhibition of the maize immunoblotting by grass, barley, wheat, and rice gave different results for the different kinds of cereal. We found that preincubation of the sera from patients allergic to maize with grass pollen, wheat, or barley extract completely inhibited the 16-kd protein and other bands with higher MWs corresponding to pro-

teins at 25, 27, 30, 32, 36, 40, 53, and 85 kd, although it did not inhibit IgE binding to the maize 9-kd allergen (Fig 3). This demonstrates that these 3 cereals have high cross-reactivity for trypsin/activated Hageman factor (XIIA) inhibitor and other proteins but not for LTP. On the other hand, rice extract completely inhibited all the allergens. In particular, the IgE binding to the 9- and 16-kd proteins

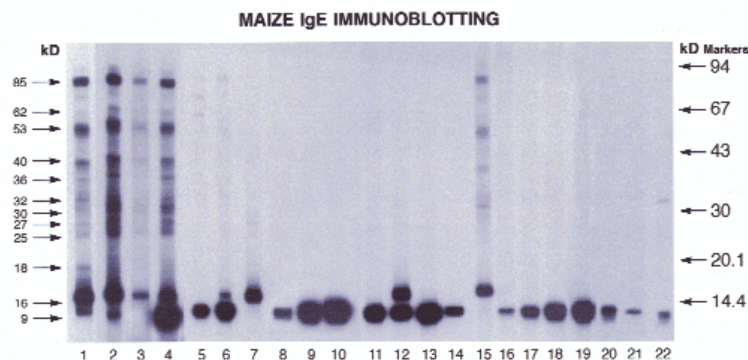


FIG 1. IgE immunoblotting of maize extract with the sera from 22 patients with severe systemic reactions to the ingestion of maize.

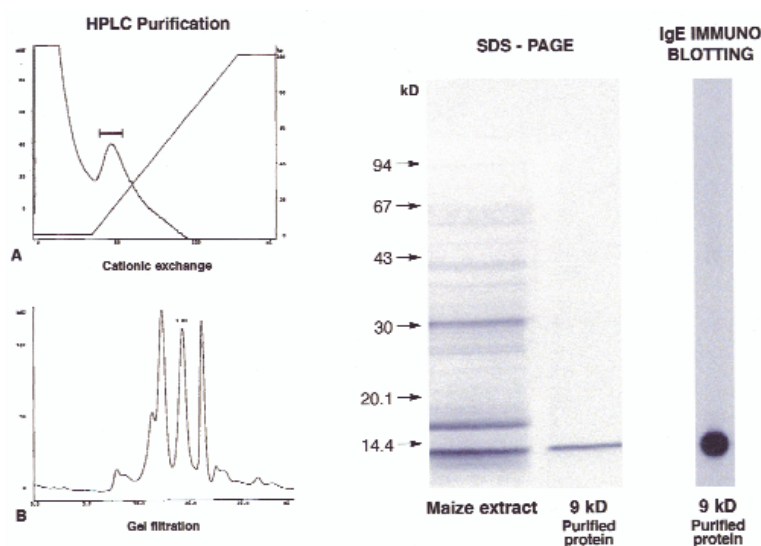


FIG 2. Left, The chromatographic profiles of maize extracts obtained from the cationic exchange column (A) and from the subsequent gel-filtration column (B). The x-axis reports milliabsorbance units (mAU) measured at a wavelength of 280 nm. The line indicates collected cationic exchange fractions used in the gel-filtration run. Right, The SDS-PAGE profiles of the crude maize extract and of the 9-kD purified protein, which correspond to the third peak from the gel-filtration passage. The 9-kD protein was also analyzed by IgE immunoblotting to reveal the purity of this fraction and its allergenic activity.

began to reappear at a protein content of 0.135 mg of rice extract. This demonstrates a total cross-reactivity between rice and maize also with regard to LTP.

Moreover, we found total cross-reactivity between maize and peach LTPs (Fig 4) because there was complete cross-inhibition, with the 9-kD bands reappearing at a 0.3-mg protein content of maize in peach immunoblotting and at a 0.483-mg protein content of peach in maize immunoblotting.

DISCUSSION

The major allergen of maize, which is responsible for provoking food-induced allergic reactions, was found to be an LTP. This protein was fully sequenced in 1988 and was shown to share with other LTPs the same low MW (approximately 9 kD), the basic characteristic (isoelectric

point between 9 and 10), and, most importantly, the presence of conserved residues, such as 8 cysteines forming 4 disulphur bridges, in the amino acid sequence.¹⁷ Its three-dimensional structure was recently deduced from roentgenographic and magnetic resonance crystallography studies.¹⁸⁻²⁰

Twenty-two patients were selected as having a history of allergic reactions to maize. These reactions were predominantly systemic, including laryngeal edema and shock, whereas only 5 patients presented with localized oral allergy symptoms.

The high physicochemical stability of the protein, conditioned by the above-mentioned disulphur bonds, and the high concentration of this protein in the maize seed and hence in maize flour could be possible explanations for such a high incidence of serious reactions: this LTP accounts for 4% of the soluble proteins in maize²¹ and is highly resistant

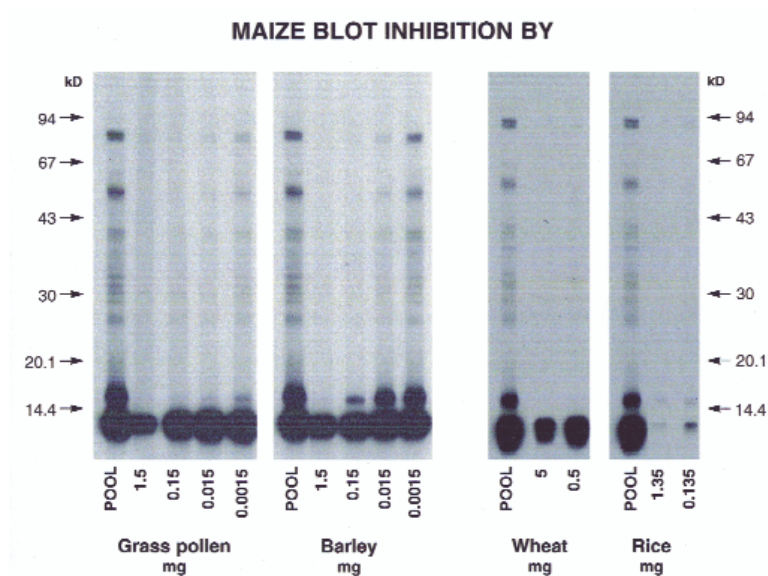


FIG 3. Inhibition of maize IgE immunoblotting (*pool*) by different concentrations of grass pollen, barley, wheat, and rice.

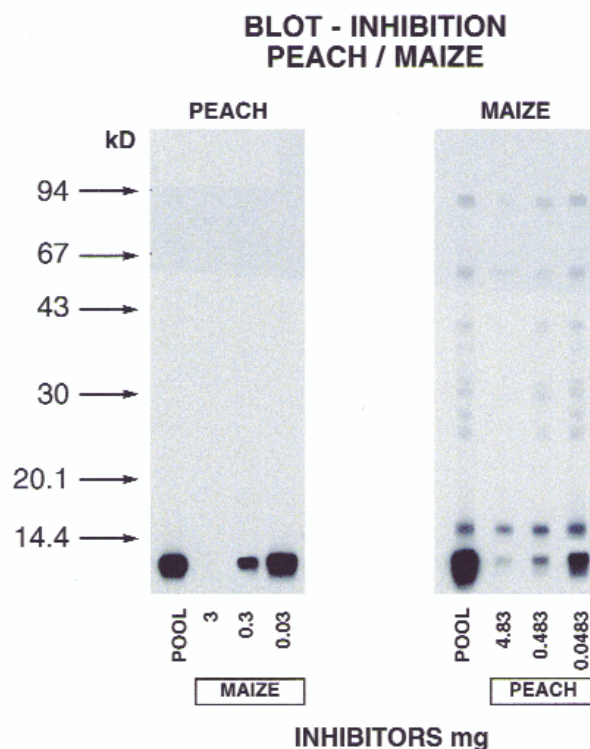


FIG 4. Inhibition of IgE immunoblotting of the maize purified 9-kd protein by peach row extract and inhibition of peach row extract IgE immunoblotting by the maize crude extract. The protein at 9 kd was completely inhibited in both experiments.

to heat and enzymatic or chemical treatment.²² Maize LTP is therefore a rapidly released and highly concentrated protein (ie, a perfect candidate for a food allergen).

Furthermore, the 9-kd LTP was found to be the only maize protein capable of sensitizing subjects not allergic to pollen because none of the 7 subjects without pollen

allergy were sensitized to other maize allergens. On the contrary, 9 of the 15 patients allergic to pollen were sensitized to other proteins of higher MWs, and 8 of these 9 reacted to the 16-kd protein, which thus proved to be the only other important allergen in maize besides LTP. Determination of its amino acid sequence revealed it to

be the inhibitor of trypsin/activated Hageman factor (XIIA), which was described in maize in 1984.²³ This is a member of the cereal trypsin and α -amylase inhibitor family, which comprises a highly homologous group of proteins with MWs ranging from 12 to 16 kd and considerable allergenic relevance. These proteins are responsible for the so-called baker's asthma, an IgE-mediated pathology elicited by inhalation of cereal flours in professionally exposed subjects.

It should be observed that at least two reports have shown that these allergens are also likely to cause clinical symptoms of food allergy in addition to respiratory allergy. Urisu et al²⁴ and Tada et al²⁵ were the first to demonstrate that the principal rice allergen is an amylase inhibitor with a MW of 16 kd, and James et al²⁶ have shown that the majority of children with positive DBPCFC results for wheat flour were sensitized to the wheat 15-kd allergen (ie, the inhibitor of trypsin/activated Hageman factor). As a confirmation of these data, our study shows that the allergen at 16 kd is an important allergen because the 3 symptomatic patients not sensitized to the maize LTP displayed IgE reactivity to this molecule, which is the main allergen in cereals and has a highly conserved structure. The immunoblotting inhibitions found results provide further confirmation. Moreover, the 16-kd allergen is possibly responsible for the in vitro cross-reactivity with grass pollen, in which it is described as Lol p 11. This protein homology has always been one of the main problems in diagnosing cereal allergy because patients with a high RAST value for grass pollen often display a false-positive RAST result for cereals without presenting any clinical allergic reaction.

The LTP was not found to cross-react with grass pollen; this is of considerable significance because the lack of cross-reactivity between grass pollens and the 9-kd maize protein implies the impossibility a high grass pollen RAST value eliciting false-positive cereal RAST results. The 9-kd LTP is thus an allergen specific of maize seeds and of maize-induced allergic reactions.

An important aspect of our study is that some of our patients presented with allergic reactions to maize exclusively as a sensitization to food, whereas others hoped to resolve their peach allergy problems. In the latter group sensitivity to maize was only revealed at a later point.

Our main reason for verifying whether maize LTP was allergenic lay in the observation of its high homology with peach LTP. Our study confirmed its being allergenic, as well as the existence of a marked cross-reactivity with peach. Fig 4 shows how peach extract completely inhibited maize blotting and vice versa. It is highly probable that the 63% homology in the amino acid sequence of the two LTPs is the underlying cause of the double sensitization to maize and peach; nevertheless, this double sensitization does not always manifest in patients allergic to one or the other of these foods.

On the contrary, there is a strikingly low cross-reactivity between maize LTP and the LTPs of other cereals. Our immunoblotting inhibition test revealed that preincubation of the serum with wheat and barley extracts did

not inhibit the binding of specific IgE to maize LTP, whereas it inhibited the bond to all other allergens with greater MWs. This explains why the majority of the patients in our study tolerated wheat flour in foodstuffs while still presenting with reactions, sometimes of a serious nature, to maize ingestion.

Rice extract, on the contrary, completely inhibited the maize blotting in all its allergenic components. Seven of the 22 patients reacted markedly to rice, and two of these (patients 7 and 20) had severe reactions (laryngeal edema). It would thus appear that maize allergy may condition a rice-induced allergic reaction, whereas its influence is considerably less with other cereals. A possible explanation derives from the comparison of the amino acid sequences of the respective LTPs: maize LTP has a very high homology with rice LTPs (79% identity and 92% similarity) and a lower homology with wheat (59%-79%) and barley (57%-78%) LTPs.²⁷

Moreover, the results of maize immunoblotting-inhibition tests carried out with other cereals demonstrate an in vitro cross-reactivity of the 16-kd (the trypsin/XIIA factor inhibitor) allergen. Further research will be necessary to elucidate which allergens are really responsible for food allergy to these cereals.

In conclusion, this study demonstrates that one of the most biologically important maize proteins, for both its defensive activity and its function in the metabolism of lipids, is also one of the major maize allergens. It is an LTP and represents the Zea m 14 allergen in the cereal group.

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