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Original article

IgE-mediated allergy to corn: a 50 kDa protein, belonging to the Reduced Soluble Proteins, is a major allergen

Background: Although corn is often cited as an allergenic food, very few studies have been devoted to the identification of corn allergens and corn allergy has been rarely confirmed by double-blind, placebo-controlled food challenge (DBPCFC). Recently, Pastorello et al. (1) identified some salt-soluble IgE-binding proteins of corn flour as potential allergens. One of these, corresponding to corn Lipid Transfer Protein (LTP), appeared to be the major one. The aim of this study was to verify the clinical significance of the skin prick test (SPT) and CAP-FEIA CAP-System IgE fluozoenzyme immunosorbent assay (Pharmacia Diagnostic, Uppsala, Sweden) positivities to corn and to identify the presence of IgE-binding proteins in the corn flour salt-insoluble protein fractions (comprising up to 96% of the total protein) using sera of patients with DBPCFC-documented food allergy to corn. In addition the effect of cooking and proteolytic digestion on the corn allergens was investigated.
Methods: Sixteen subjects with SPT and CAP-FEIA positivities to corn flour were examined. Only six of them complained of suffering from urticaria and/or other symptoms after ingestion of corn-based foods. The patients were food challenged with cooked corn flour (polenta). IgE-binding proteins were detected by immunoblotting. The digestibility of the IgE-binding proteins was examined during a pepsin attack followed by a pancreatin digestion performed on a cooked corn flour sample.
Results: Oral challenge was positive only for six patients with symptoms after ingestion of corn. A 50 kDa protein, belonging to the corn Reduced Soluble Protein (RSP) fraction was recognized by the serum IgE of all the DBPCFC-positive subjects and resulted to be resistant to both heating and peptic/pancreatic digestion. SPT with the purified RSP fraction gave positive results for all of the DBPCFC-positive patients examined.
Conclusions: SPT and CAP-FEIA positivities to corn flour had no clinical significance for most of the patients and food allergy to corn has to be proved by DBPCFC. A salt-unextractable protein of 50 kDa, belonging to the RSP fraction, represents a potential allergen in food hypersensitivity to corn because of its stability to cooking and digestion.

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Corn (*Zea mays* L.) is the basic ingredient of frequently eaten foods like porridge, polenta, cornflakes, popcorn, taco chips, canned de-embryonated kernels, and so on. Moreover, corn is used in the formulation of various food products such as bread, cakes and beer. As a flour, corn is also inhaled by people handling it while making cakes (bakers, pastry cooks, housewives) or making feed (farmers).

Normal corn flour contains about 7–13% protein that can be fractionated into several solubility classes. The salt-extractable fraction (albumins and globulins), averaging less than 4% of the total endosperm protein (2), mainly comprises proteins with metabolic functions. In contrast, extraction with aqueous alcohol results in the solubilization of the prolamin fraction that contains the storage proteins of the corn seed. These latter, constituting about 60–70% of the corn endosperm proteins (2, 3), are called zeins and comprise various polypeptides differing in molecular weight and isoelectric points (4), and are classifiable into four major groups (α, β, γ, and δ zeins) on the basis of their molecular characteristics (5). Some proteins extracted with reducing alcoholic solutions, however, are also soluble in water in reducing conditions and for this reason are called Reduced Soluble Proteins (RSP), making questionable their identification as true zeins (3, 6). Finally, a heterogeneous alcohol-insoluble protein fraction exists in the corn flour called glutelin fraction (3). This fraction can be extracted only with strong denaturing solvents (4).

Some recent papers have reported cases of allergic respiratory reactions (7) to inhaled corn dust in exposed workers and corn components binding to IgE have been identified (8, 9). Heat shock protein-related epitopes have been reported to be common allergic determinants for corn antigens in a population of pig farm workers exposed to complex bioaereosols (10).

It is very surprising that, although corn is often cited as an allergenic food and RAST positivities to this cereal have been reported, cases of allergic reactions after ingestion of corn have been rarely published and very few studies have been devoted to the identification of corn allergens. On the basis of a retrospective study on patients with histories of food allergy Moneret-Vautrin et al. (11) concluded that food allergy to corn is rare. A case of food-dependent exercise induced anaphylaxis to corn (12) and a few cases of corn allergy in children have been reported (13), although the allergens involved have not been investigated. Lehrer et al. (14) found significant levels of IgE to corn water-soluble and insoluble proteins in the sera of 'corn-reactive individuals', but the clinical manifestation of such corn-reactivity was not reported. Recently, Pastorello et al. (1) have identified some salt-soluble IgE-binding components of corn flour: a 9 kDa protein, corresponding to the corn Lipid Transfer Protein (LTP) and a 16 kDa protein, corresponding to a trypsin inhibitor, were bound by the IgE of 86% and 36% of the examined patients, respectively.

In this paper we have examined a population of subjects with SPT and CAP-FEIA positivities to corn flour and we have proved that only a minor fraction of these individuals had a true food allergy to corn. Moreover the potential allergens have been identified by immunoblotting, and the effect of both cooking and proteolytic digestion on them has been studied.

Material and methods

Extraction proteins from corn flour

We extracted 5 g of commercial corn flour with 25 ml of 0.5 M NaCl for 2 h at 4°C. After centrifugation (12 000 *g*, 20 min, 4°C) the supernatant containing the salt-extractable proteins (SEP) was collected and stored at −20°C for subsequent use. The residue was washed with 50 ml of 0.5 M NaCl and centrifuged again. This procedure was repeated and a final wash with distilled water was done. The SEP-free residue was then extracted overnight with 25 ml of 80 mM Tris-HCl, pH 8.5, 50% (v/v) propan-2-ol, containing 2.5% (v/v) 2-mercaptoethanol (2-ME) at room temperature with continuous stirring. After centrifugation (12 000 *g*, 20 min, 4°C) the supernatant (alcohol-extractable proteins, AEP) was collected and stored at −20°C.

In other experiments, the RSP were extracted from the SEP-free residue with 50 ml of 50 mM Tris-HCl, pH 7.4 containing 20 mM dithiothreitol, as described by Vitale et al. (6).

Total corn proteins were extracted from 100 mg of flour with 4 ml of 100 mM Tris-HCl buffer, pH 8.3, containing 6 M urea, 2.5% (w/v) sodium dodecyl sulfate (SDS), 2.5% (v/v) 2-ME and 10% (w/v) glycerol and heated for 5 min at 100°C. After centrifugation (12 000 *g*, 20 min), the supernatant (total protein extract), was collected and stored at −20°C.

Cooking of corn flour

One hundred grams of corn flour were suspended in water (1 : 10) and cooked for 30 min with continuous mixing, producing a gelatinous gruel. After cooling at room temperature the cooked sample was freeze-dried and re-ground. The different protein fractions, SEP, AEP, and RSP, and the total protein extract were obtained as reported above for the raw flour.

Protein digestion

Sixty milligrams of freeze-dried cooked corn flour was suspended in 4 ml of 0.2 N HCl (pH 2.0), containing 0.05 mg/ml of pepsin (E.C. 3.4.23.1; pepsin from hog stomach ≈3000 U/mg protein, Fluka (Milan, Italy)). A control (undigested) sample was treated in the same manner, but without pepsin. Proteolysis was carried out at 37°C in a shaking water bath for 30 min. Samples were then added of 1.15 ml of 1 M boric acid, 0.5 N NaOH, adjusted to pH 6.8 with 5 N HCl and containing 0.25 mg/ml of pancreatin (from porcine pancreas, Sigma (Milan, Italy)).The resulting pH was 7.6 (15). The reaction was stopped at different times (0, 15, 30 min of pepsin attack and 15, 30, 60, 120 min of pancreatic digestion) by addition of 0.5 volumes of 0.3 M Tris-HCl, pH 8.3, containing 20% (w/v) glycerol, 7.5% (w/v) SDS and 7.5% (v/v) 2-ME. Samples were immediately heated in a boiling water bath for 10 min After cooling and centrifugation, the supernatants were stored at −20°C.

Electrophoresis

SDS-PAGE was performed according to Laemmli (16) in a Mini Protean II cell (Bio-Rad, Milan, Italy). Gels were prepared with a total polyacrylamide concentration of 18%. Before electrophoresis, the different protein fractions were processed as follows. The SEP and RSP solutions were diluted with 0.25 volumes of a 1 M Tris-HCl buffer, pH 7.4, containing 8% (w/v) SDS, 5% (v/v) 2-ME, 40% (w/v) glycerol and heated for 5 min in a boiling water bath. The AEP were precipitated from 1 ml of solution by addition of 9 ml of cold acetone. The dried pellet was solubilized with 1 ml of 0.2 M Tris-HCl, pH 7.4, containing 2% (w/v) SDS, 5% (v/v) 2-ME, 8% (w/v) glycerol and heated for 5 min in a boiling water bath. Total protein extracts were only heated as above. Electrophoresis was run at 50 mA constant current until the tracking dye bromophenol blue reached the bottom of the gel. Digested samples taken at the different times were analyzed by tricine-SDS-PAGE (T-SDS-PAGE) according to Schägger and von Jagow (17) in a 16% total polyacrylamide gel. Before electrophoresis samples were heated for 5 min in a boiling water bath.

Gels were stained with Coumassie brilliant blue or used for blotting. Molecular weight standard proteins (Bio-Rad) were phosphorylase B (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (45 kDa), carbonic anhydrase (31 kDa), soy trypsin inhibitor (21.5 kDa) and lisozyme (14.4 kDa).

Immunoblotting

IgE immunoblotting with individual or pooled patients' sera was performed as previously described (18).

Specific IgE detection and skin prick tests

The detection of specific serum IgE to corn flour was performed with the ImmunoCAP-system (CAP-System-FEIA, Pharmacia Diagnostic, Uppsala, Sweden). The results were expressed as CAP scores from class 0 to 6, according to the manufacturer's instructions. SPT were performed using commercial allergens (Bayer, Milan, Italy) to the common inhalants and foods, including corn flour. SPT were also carried out for an in-house corn flour extract (in saline phosphate) and the RSP fraction. Readings were made at 15 min; wheal

diameters equal to or larger than 3 mm were considered as positive in the absence of a reaction to the negative control. In all cases the informed consent of the patient was obtained.

Double-blind, placebo-controlled food challenge

DBPCFCs were performed using a special meal prepared as follows: corn flour was milled into a fine powder (25 g) and boiled in salted tap water (100 ml) for 30 min with continuous mixing (polenta). Peeled potatoes (25 g), carrots (10 g), and turmezic (1/4 coffee spoon) were cooked, mashed with a blender, added to polenta and mixed. A tablespoon of olive oil was added. The placebo meal was prepared by substituting the corn flour with two tablespoons of tapioca flour and increasing the quantity of carrot to 20 g.

The patients were first challenged with the placebo meal in order to exclude any reaction to the ingredients used. Then, they were challenged in an open way with the corn-containing meal. The patients first ate three tablespoons of meal. If no symptoms appeared after 1 h, they ate two additional portions of four tablespoons at 1 h intervals. Thereafter only the patients that had reported some reaction to the ingested meal were subjected to DBPCFC. Patients were kept under observation for 3 h and the appearance of symptoms during this time, such as oral allergy syndrome, rhinoconjunctivitis, wheezing, itching, angioedema and urticaria, were recorded.

Results

Clinical history of the patients

Table 1 summarizes the characteristics of the patients examined in this study. All patients were SPT and CAP-FEIA positive to corn. All of them suffered from allergic respiratory symptoms to inhalants. Six patients (patients 1–6) referred to suffering from urticaria and other symptoms after ingesting corn-based foods (such as polenta), requiring in some cases immediate hospitalization and treatments with corticosteroids. The others (patients 7–16) claimed that eating corn-based foods was safe for them. All patients tested, except for three, were CAP-FEIA positive to grass and corn pollens.

DBPCFC with cooked corn was positive for patients 1–6. All these patients experienced itching and urticaria during the challenge, these symptoms appearing within 120 min The other patients (patients 7–16), who responded negatively to the open challenge with the corn-based meal, were not submitted to DBPCFC.

SDS-PAGE and immunoblotting with raw corn proteins

Corn salt-extractable proteins (SEP, albumins and globulins) and alcohol/2-ME-extractable proteins (AEP) were separated by SDS-PAGE (Fig. 1A, lanes 1 and 2, respectively). The patterns of the two protein classes were clearly different, SEP showing a higher number of bands distributed in a broad molecular weight range. The AEP pattern comprised some heavy stained bands with molecular weights of between 14 and 31 kDa corresponding to zein polypeptides, the storage proteins of the corn seed (5). In addition, other bands were present with molecular weights of around 50 kDa; these bands were not extractable when the reducing agent was omitted from the alcoholic buffer (not shown), confirming previously reported results (6). When the total protein extract was analyzed by SDS-PAGE (lane 3), the major zein bands and some bands of higher molecular weight, probably glutelin components (3), were detected, whereas most of the bands corresponding to the soluble proteins were undetectable. This should be due to the very low relative quantity of the SEP, which represents less than 4% of the total protein of the corn endosperm (2).

The single sera of five of the DBPCFC–positive

Table 1. Allergological features of the subjects

Patients	Symptoms after corn ingestion[a]	Corn Tests[b]			CAP positivity to inhalants[c]
		CAP value	SPT	Challenge	
1	U,GI	3	Pos.	Pos.	Gr, Ma, Df, Dp
2	U,AE	4	Pos.	Pos.	Gr, Ma, Co, Ol
3	U,OAS	4	Pos.	Pos.	Gr, Ma, Co
4	U,OAS	2	Pos.	Pos.	–
5	U	3	Pos.	Pos.	Df, Dp
6	GI,A,RC	4	Pos.	Pos.	Gr, Ma, Co
7	None	4	Pos.	Neg.	Gr, Co, Be
8	None	2	Pos.	Neg.	Gr, Ma, Co
9	None	3	Pos.	Neg.	Df, Dp
10	None	2	Pos.	Neg.	Gr, Ma, Pa
11	None	3	Pos.	Neg.	n.d.
12	None	3	Pos.	Neg.	Gr, Ma, Pa, Co
13	None	2	Pos.	Neg.	Gr, Ma, Be
14	None	2	Pos.	Neg.	Gr, Ma
15	None	4	Pos.	Neg.	Gr, Ma
16	None	3	Pos.	Neg.	Gr, Ma, Df, Dp

[a]A: asthma; AE: angioedema; GI: gastrointestinal symptoms; OAS: oral allergy syndrome; RC: rhinoconjunctivitis; U: urticaria.
[b]Pos: positive; Neg: negative.
[c]Be: birch pollen. Co: Compositae mix pollens. Df: Dermatophagoides farinae. Df: Dermatophagoides pteronissimus; Ma: corn pollen. Pa: Parietaria pollen.
n.d.: not determined.

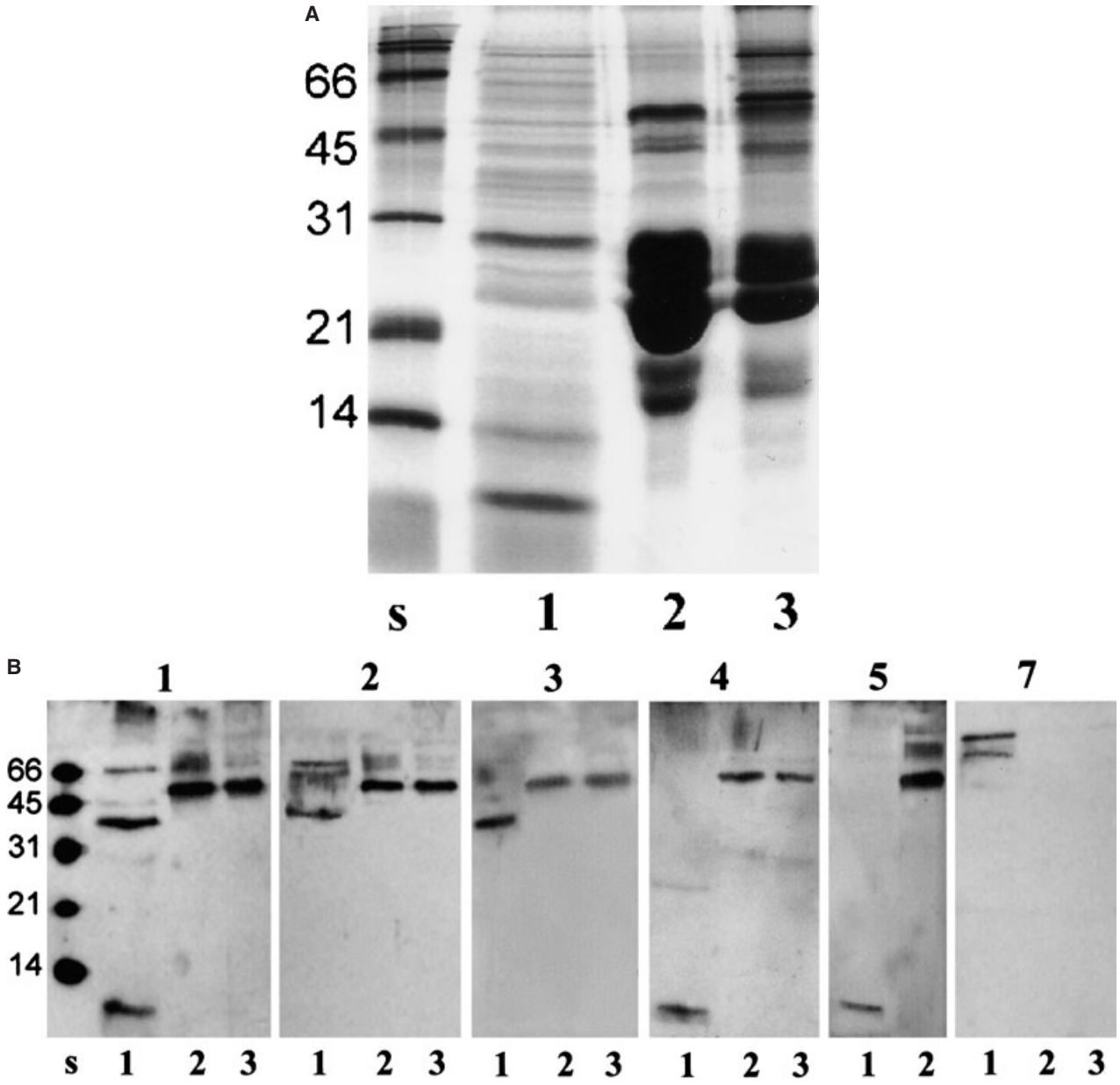

Figure 1. SDS-PAGE analysis of corn flour proteins. *A)* Coumassie brilliant blue stain. *B)* IgE immunoblot with single patients' sera (numbers on the top correspond to patients in Table 1). Lanes 1: salt-extractable proteins (SEP); lanes 2: alcohol/2mercaptoethanol-extractable proteins (AEP); lanes 3: total proteins. Molecular weight standard proteins are in lanes labelled s.

patients (patients 1–5) were examined in IgE immunoblotting experiments. All of them revealed IgE-binding to SEP bands (Fig. 1B, lane 1). Three out of five of these patients (patients 1, 4 and 5) had IgE-binding to a soluble protein of about 9 kDa, which is likely to correspond to the LPT of the corn endosperm (1). The sera of one subject (patient 7) who did not suffer from corn allergy also recognized some SEP bands (Fig. 1B), whereas no bands were recognized by the serum of a healthy individual (not shown).

All the sera of the DBPCFC-positive patients examined had IgE that also recognized a component of about 50 kDa that was present in the AEP fraction (Fig. 1B, lane 2), whereas no binding to the predominant zein bands was observed. When the total extract was analyzed, only the 50 kDa band was recognized by the IgE of the corn allergic patients (Fig. 1B, lane 3). The sera of the control subject (patient 7, Fig. 1B, lanes 2 and 3) and of the healthy subject (not shown) did not contain IgE to bands of the insoluble corn protein fractions. Due to its molecular weight and solubility properties, the 50 kDa IgE-binding band present in both the AEP and the total protein extract was suspected to belong to the RSP fraction (6). In order to confirm this fact, the RSP [which have been reported to be soluble in both alcohol and acqueous buffers, but

only under reducing conditions (6)] were selectively extracted from the SEP-free residue by a dithiotreitol-containing buffer, as described by Vitale et al. (6) and analyzed by SDS-PAGE and IgE immunoblotting. Among the different protein bands of the RSP fraction revealed by SDS-PAGE (Fig. 2A), that with the molecular weight of 50 kDa was actually recognized by the IgE of the sera of all DBPCFC-positive patients tested (Fig. 2B).

SPT with corn RSP fraction

The RSP fraction was tested by SPT in four of the DBPCFC-positive patients (patients 1, 2, 3 and 6). All the patients reacted positively to the RSP fraction (data not shown).

SDS-PAGE and immunoblotting with cooked corn before and after *in vitro* digestion

Since corn induced the allergic response when ingested after being cooked, the effect of both cooking and proteolytic digestion on the IgE-binding proteins of the corn flour was considered. The electrophoretic pattern of the SEP extracted from the cooked corn was different from that of the raw corn (Fig. 3A). In fact, many protein bands, including that of 9 kDa, were not detectable by Coumassie brilliant blue staining in the SEP extract from the cooked corn (compare lanes 1 and 2). As already pointed out (19), most of the SEP cannot be solubilized from a cooked corn sample by the salt solution used for their extraction from the raw flour. In contrast, the electrophoretic patterns of the RSP fractions from raw and cooked corn were rather similar (lanes 3 and 4). In particular, the 50 kDa protein band was evident also in the extract obtained from the cooked corn.

Immunoblotting experiments with pooled sera of DBPCFC-positive patients (patients 1–5) revealed that the IgE-binding pattern of the proteins extracted by salt from the cooked sample was different from that obtained with the raw one (Fig. 3B). The differences in the IgE-binding pattern indicated that some physicochemical modifications of the allergens occurred during cooking of the corn flour. In particular, in the cooked sample no IgE-binding was observed in the gel region corresponding to the 9 kDa protein band (lane 2). However, IgE-binding to the band corresponding to the 50 kDa allergen belonging to the RSP fraction was maintained in the cooked sample, showing that the physicochemical features and the IgE-binding ability of this protein were not affected by the heat treatment (lanes 3 and 4).

The digestibility of the corn IgE-binding proteins was examined during a pepsin attack followed by a pancreatin digestion performed on a cooked flour sample. The total proteins of the corn flour at the different digestion times were analyzed by Tricine-SDS-PAGE (Fig. 4), an electrophoretic technique allowing detection of proteolytic fragments with very low molecular weights (17). IgE immunoblotting with pooled sera revealed that the 50 kDa allergen was stable during 30 min of pepsin degradation and resisted to the pancreatic enzymes for a rather long time, disappearing only after more than 90 min of pancreatin treatment (Fig. 4B). Other IgE-binding proteins (i.e. those of the SEP) were not detectable in the undigested and digested corn samples because, as reported above, their amount was too low in a corn flour total protein extract.

Discussion

DBPCFC tests with cooked corn flour confirmed that the symptoms showed by six out of the 16 patients examined in this study were clinical manifestations of an IgE-mediated hypersensitivity reaction to ingested corn. Although also the other 10 patients considered were positive to both SPT and CAP-FEIA to corn, they were not suffering from food allergy to this cereal as revealed both by their clinical history and the negative challenge results. Our findings confirm the observations of Jones et al. (20), who found that only five of 17 subjects with positive SPT to corn reacted to an oral challenge with this cereal. These authors, because of the high incidence in the examined population of allergy to pollen of grasses (which are taxonomically related to corn), concluded that clinically insignificant cross-reactivity exists among cereal grains and grasses. Our results confirm this conclusion, since most of our patients were suffering from respiratory allergy to grass pollens (as well as to corn pollen) without any relationships with the results of the food challenge.

Pastorello et al. (1), examining the soluble protein fraction of the corn flour, identified some IgE-binding components by immunoblotting with sera of patients who reported adverse reactions after eating corn. A 9 kDa protein, identified as the corn LTP, and a 16 kDa protein corresponding to a trypsin inhibitor were bound by the IgE of 86% and 36% of the patients, respectively. A 10 kDa protein corresponding to the barley LTP has been found to be recognized by the IgE of patients allergic to beer (18). The results reported here confirm that IgE-binding to a corn protein with a similar molecular weight are present in the sera of some patients with documented food allergy to corn. However, the salt-soluble fraction comprises only a minimal quantity (less than 4%) of the total corn proteins, whereas most of the proteins are extractable with aqueous alcohol in reducing conditions (2, 3). For this reason, the presence of potential allergens in the corn alcohol-soluble fraction has been investigated. We have found that an insoluble 50 kDa protein was bound by the IgE of all the DBPCFC-positive patients examined

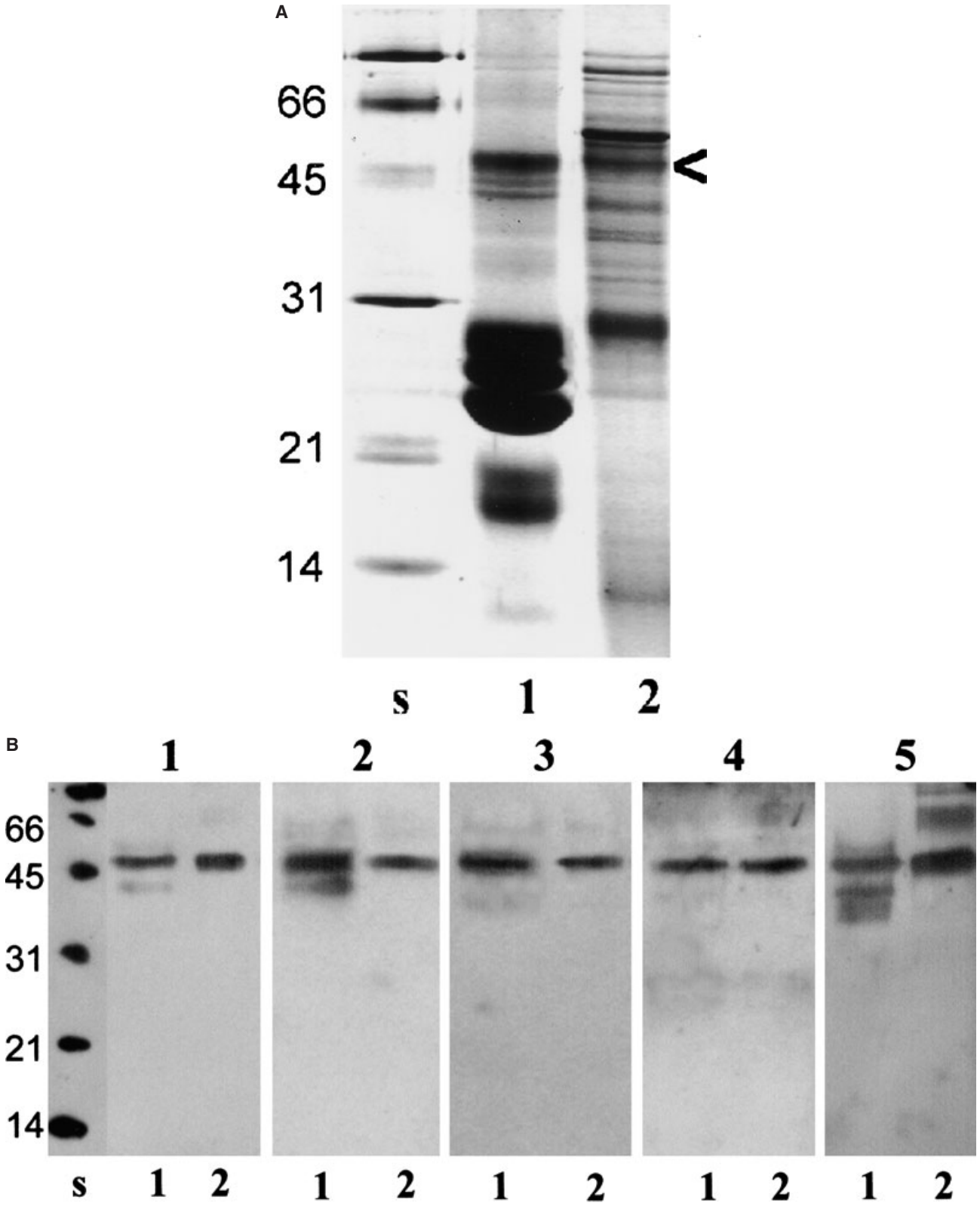

Figure 2. SDS-PAGE analysis of corn flour proteins. *A)* Coumassie brilliant blue stain. *B)* IgE immunoblot with single patients' sera (numbers on the top correspond to patients in Table 1). Lanes 1: alcohol/2-mercaptoethanol-extractable proteins; lanes 2: Reduced Soluble Proteins (RSP). The arrowhead in part A indicates the 50 kDa IgE-binding band. Molecular weight standard proteins are in lanes labelled s.

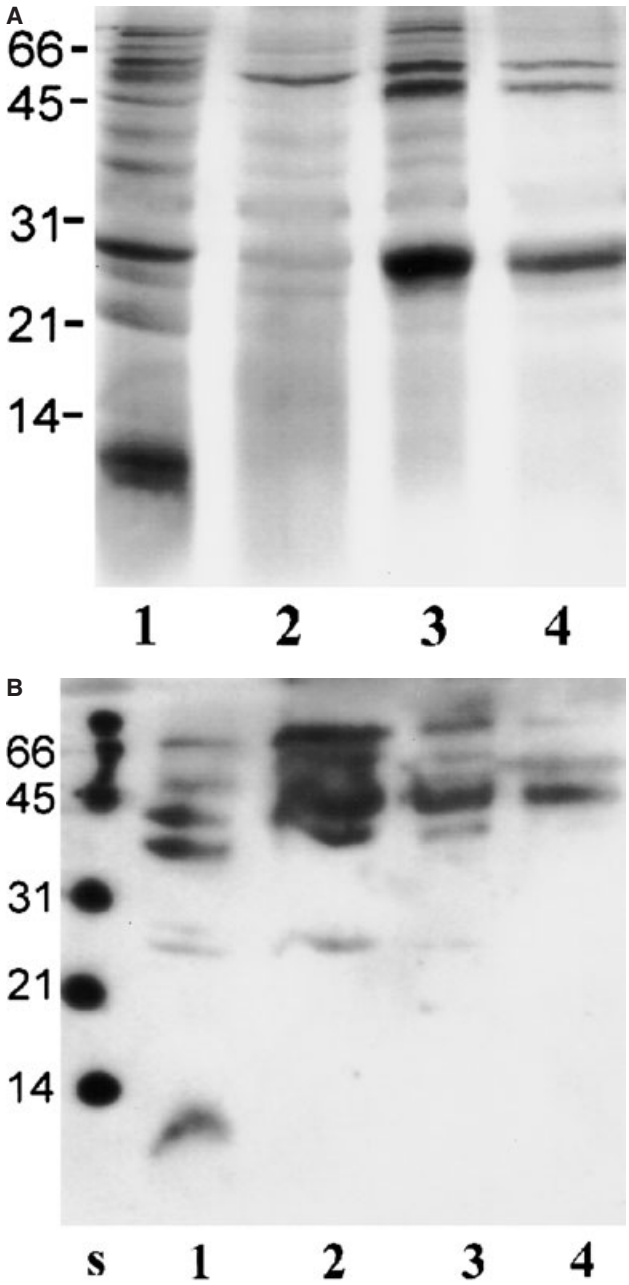

Figure 3. SDS-PAGE analysis of the proteins extractable from cooked corn flour. *A)* Coumassie brilliant blue stain. *B)* IgE immunoblot with pooled sera of corn allergic patients (patients 1–5 in Table 1). Lanes 1 and 2: salt-extractable proteins of raw and cooked corn flour, respectively. Lanes 3 and 4: Reduced Soluble Proteins extractable from raw and cooked corn flour, respectively. Molecular weight standard proteins are in lanes labelled s.

by immunoblotting. Since this protein could be also extracted with water, but only in reducing conditions, we conclude that it belongs to the RSP fraction of the corn endosperm (6). The allergenicity of this latter protein fraction was confirmed by SPT. Although further studies are needed to precisely characterize this 50 kDa corn protein, our results demonstrate that a salt-insoluble protein can act as allergen in the case of allergy to ingested corn. IgE-binding to insoluble proteins has been reported also in wheat, using sera of patients suffering from either baker's asthma (21), or from food allergy to wheat (22, 23). The IgE-binding components were identified as corresponding to members of the wheat prolamins (gliadins and glutenin subunits). However, in the corn flour, we have not observed any IgE-binding to the main zein components, which are the predominant prolamins of the corn seed.

Most of the studies on food allergy are focused on allergens that are soluble in water/salt solutions. On the basis of the results reported here, and considering that the solubility characteristics of a given protein can be remarkably changed during the digestion process, we

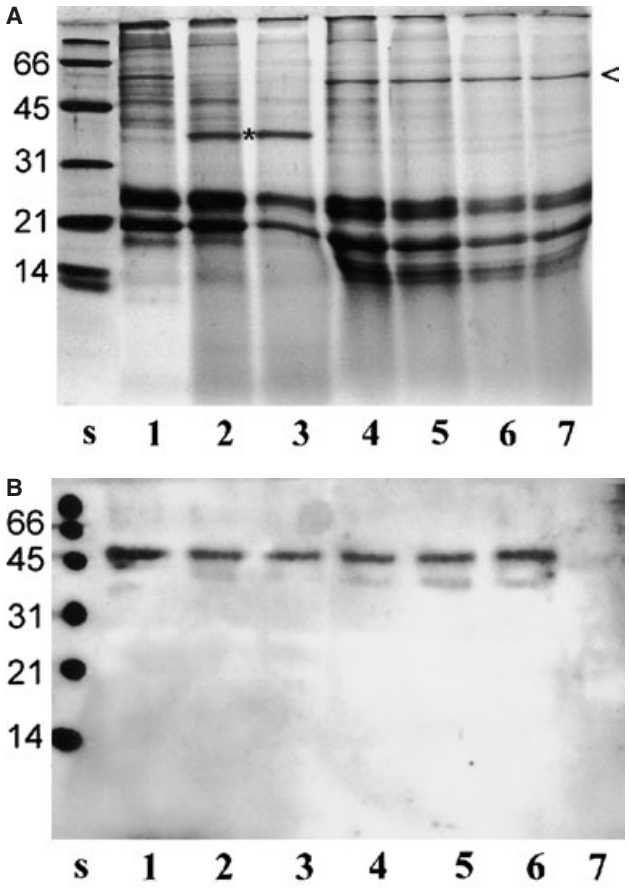

Figure 4. A) SDS-PAGE analysis of the digestion products of cooked corn flour. Coumassiee stain. *B)* IgE immunoblot with pooled sera of corn allergic patients (patients 1–5 in Table 1). Lane 1: undigested sample; lanes 2 and 3: samples after 15 and 30 min of pepsin digestion, respectively; lanes 4, 5, 6 and 7: 30 min pepsin-digested samples after 15, 30, 60 and 120 min of pancreatin digestion, respectively. The asterisk and the arrowhead in part A indicate bands belonging to the pepsin and pancreatin preparations, respectively. Molecular weight standard proteins are in lanes labelled s.

recommend performing studies on food allergen identification by considering also the insoluble proteins of raw materials used for preparation of the allergenic food.

Studies on food allergy are commonly carried out by using raw materials as sources of potential allergens, also when the incriminated foods are eaten after cooking. Heat treatments can have important effects on the allergenic potential of processed foods. They can either reduce or destroy protein allergenicity or give origin to new allergens by physicochemical modifications of proteins that are inoffensive in their native state (24). Therefore, the direct effect of cooking on the allergenicity of those foods that are consumed after being cooked should be carefully considered. Our results demonstrate that, whereas most of the soluble IgE reactive proteins of the raw flour seem to be modified after cooking, the insoluble 50 kDa allergen is not affected by the heat treatment, neither in terms of its solubility and electrophoretic features nor for its binding to serum IgE.

Moreover, cooking can modify protein digestibility and then affect indirectly the form in which the ingested allergens interact with the organism (25). For this reason, *in vitro* digestion experiments on cooked foods are of relevant interest in the cases of food allergies in which the allergens are thought to act or to be absorbed at the level of the gastrointestinal tract (26). It is commonly assumed that proteins that are susceptible to be completely degraded during digestion are inherently safer, especially in relation to food allergy, than those that are more resistant to proteolysis (27). On the basis of these considerations it is widely accepted that the ability of food allergens to reach the intestinal mucosa is a prerequisite to allergenicity (28, 29), although the effect of cooking on this latter characteristic has been rarely considered. The data reported here demonstrate that the insoluble 50 kDa allergen of the corn flour is quite resistant to both gastric and pancreatic digestion in the form in which it is consumed (in a cooked sample). Taking into account that this protein is cross-linked through covalent (S–S) bonds in the flour (6), its characteristic stability could be due to the presence of such bonds. Therefore the factors determining the insolubility of this protein are also likely to be involved in its resistance to both heating and proteolysis.

In conclusion, the insoluble 50 kDa protein present in the RSP fraction of the corn flour is a candidate to act as an allergen in IgE-mediated food allergy to corn products.

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