

Allergens, IgE, mediators, inflammatory mechanisms

Immunologic cross-reactivity among cereal grains and grasses in children with food hypersensitivity

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Background: Because of a lack of clinical data from food challenges, allergists often recommend dietary restriction of all cereal grains in patients with sensitivity to at least one grain.

Objectives: The purposes of this study were to assess the degree of intrabotanical cross-reactivity among cereal grains and related grasses, to better define the prevalence of multiple grain hypersensitivity, and to define the protein fractions associated with wheat hypersensitivity.

Methods: One hundred forty-five patients evaluated by food challenges and skin prick tests were divided into three groups: group 1, cereal grain and grass allergies; group 2, wheat allergy alone; and group 3, grass allergy alone. Fifteen patients were further selected from groups 1 to 3. Sodium dodecylsulfate-polyacrylamide gel electrophoresis and immunoblot analyses were performed on six grains and four related grasses with sera from these patients.

Results: Only 21% of patients had symptomatic reactivity as determined by food challenge; 80% had reactivity to only one grain. As determined by immunoblot analyses, patients in groups 1 and 2 showed extensive cross-reactivity (within each group) among grains but little cross-reactivity among grasses, whereas patients in group 3 showed cross-reactivity between the grains and grasses. Patients with wheat allergy had specific IgE binding to wheat fractions 47 kd and 20 kd, bands not recognized by patients with grass allergy.

Conclusions: Clinically insignificant cross-reactivity exists among cereal grains and grasses; therefore, elimination of all grains from the diet of a patient with grain allergy is unwarranted. Further purification and characterization of the 47 kd and 20 kd wheat fractions is needed to provide more specific *in vitro* testing. (*J ALLERGY CLIN IMMUNOL* 1995;96:341-51.)

Key words: Cereal grains, grasses, wheat, food challenges, cross-reactivity, immunoblot

Cereal grains constitute the staple food for most of the world's population, providing approximately half of the world's supply of human dietary protein. The annual worldwide production of wheat, rice, and corn averages nearly 300 kg per person.¹ According to the Food and Agriculture Organization of the United Nations, 1.87 billion tons of cereal grain were produced in 1989 alone.² The list of foods and food uses for cereal grains is enormous. Cereal grains are used as thickening agents, fillers, and binders; and

Abbreviations used

DBPCFC:	Double-blind, placebo-controlled food challenge
MWMs:	Molecular weight markers
PBS:	Phosphate-buffered saline
PBST:	PBS plus 0.05% Tween-20
PBST gel:	Porcine gelatin in PBS, pH 7.4, with 0.05% Tween-20
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
SPT:	Skin prick test

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they can be found in baked goods, processed meat products, confectionery items, drinks such as malted milk, and many other processed foods.

The term *cereal* implies a functional rather than taxonomic meaning, with cereals being the most

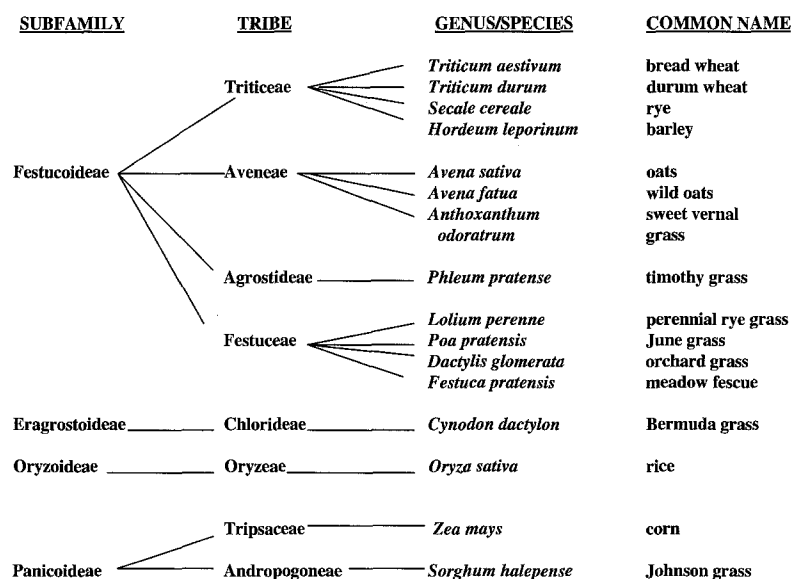


FIG. 1. Taxonomic relationship between cereal grains and grasses of family Poaceae.

important members of the grass species. Cereal grains and various grasses are monocotyledons and are taxonomically classified in the Poaceae family (Fig. 1).¹

For many years cereal grains have been recognized as the cause of adverse reactions in some human beings. Several primary disease processes have been recognized in association with wheat exposure: gluten-sensitive enteropathy (celiac disease), baker's asthma, and generalized food hypersensitivity. Celiac disease is a non-IgE-mediated enteropathy caused by the gliadin fraction of wheat.^{3,4} Baker's asthma has been well studied and demonstrated to be an IgE-mediated allergic reaction to inhaled flour (wheat and other cereal grains), primarily seen in patients with occupational exposure.⁵ Patients with asthma caused by inhaled flour, however, virtually never have problems with ingested cereal grains. The third hypersensitivity category is related to ingestion of cereal grains, that is, IgE-mediated food hypersensitivity. Allergic reactions to foods, consisting of cutaneous, respiratory, and gastrointestinal symptoms, have been reported by many investigators.^{1, 6-11}

On the basis of these observations, investigators have attempted to evaluate patients suspected of having food hypersensitivity to cereal grains with in vivo and in vitro testing, such as skin prick tests (SPTs) and RASTs.^{4, 9, 11-13} Many report high circulating levels of wheat-specific IgE antibodies in these patients. Most also note that RAST positivity frequently does not correlate with clinically significant food hypersensitivity.^{4, 11, 12} Furthermore, in vitro evaluations have been performed in patients

with food hypersensitivity and in patients with baker's asthma to evaluate the degree of related grass allergy in these patients. Investigators have observed increased positive RAST results to grasses in patients with circulating cereal grain-specific IgE and baker's asthma. They have questioned the clinical relevance of these findings, suggesting that cross-reactivity may occur, resulting in false-positive in vitro test results to grass pollens.*

Because of a lack of clinical data from controlled food challenges, allergists often recommend strict dietary elimination of all cereal grains in patients with sensitivity to at least one grain. This practice is questionable for several reasons. First, studies evaluating children with food hypersensitivity to legumes or fish species have shown extensive in vitro cross-reactivity but little clinical cross-reactivity among members of these particular groups.¹⁶⁻¹⁸ Second, large-scale studies in which double-blind, placebo-controlled food challenges (DBPCFCs) were used to diagnose food allergy have shown that 80% of children react to only one or two foods.^{19,20} Third, excessive dietary restrictions in children may lead to malnutrition and growth failure.^{21, 22}

In view of these issues and the fact that cereal grains are believed to be important causes of food hypersensitivity, we elected to study patients with suspected food hypersensitivity to cereal grains by using a combination of in vitro testing and inpatient food challenges. The four aims of this study were: to assess the degree of intrabotanical cross-

*References 1, 4, 7, 9, 11, and 13-15.

reactivity in vitro and in vivo among cereal grains; to assess the degree of in vitro cross-reactivity with other taxonomically related species (i.e., grasses); to better define the prevalence of wheat hypersensitivity in our population; and to define the particular protein fractions that are most closely associated with symptomatic wheat hypersensitivity. More precise antigen characterization may ultimately lead to improved in vitro diagnostic tools and immunotherapeutic approaches.

METHODS

Subjects

One hundred forty-five patients with positive SPT responses to one or more cereal grains (wheat, rye, barley, oat, rice, corn) were included in our cereal grain hypersensitivity study. All 145 patients were evaluated with food challenges. Diagnosis of allergy to grasses was determined on the basis of positive SPT responses, and allergy to cereal grains was determined on the basis of positive food challenge results. Patients with allergic disease were further divided into three groups: group 1, patients with cereal grain and grass allergy ($n = 15$); group 2, patients with wheat allergy alone ($n = 8$); and group 3, patients with grass allergy alone ($n = 47$). Seventy-five patients were excluded from the study because of negative food challenge results or lack of complete grass SPT response. From groups 1 to 3, 15 patients were also selected (based on availability of large quantities of serum) to evaluate specific antibody sensitivity to cereal grains and grasses. Four patients were selected from group 1, five patients from group 2, and six patients from group 3. Patients with celiac disease were excluded from the study. Informed consent was obtained from each patient, as specified in the guidelines established by the Johns Hopkins Institutional Review Board on Human Investigation.

Food challenges

All patients were admitted to the Pediatric Clinical Research Unit for oral food challenge studies. Patients were instructed to discontinue all antihistamines 7 to 10 days before admission and oral steroids 1 month before admission. Patients were asked to adhere to a strict elimination diet of the suspected food allergen for 10 to 14 days before admission. Patients were challenged with specific cereal proteins as indicated by history and SPT results, except for those with a "convincing history" of anaphylaxis to an isolated ingestion of the suspected food. Anaphylaxis was defined as a potentially life-threatening IgE-mediated event occurring within 1 hour of the ingestion of a suspected food and requiring emergency treatment and evaluation. Challenges were performed as previously described.²³ Briefly, one DBPCFC was performed each day, one with placebo and the other with the test food. Up to 10 gm of the challenge substance was administered under observation in graduated doses in a juice or "safe" food vehicle

during a 90-minute period. Symptoms were recorded in a standardized fashion during each challenge. All positive challenges were halted with the onset of objective adverse symptoms. All negative blinded challenges were confirmed with open challenges before discharge.

SPTs

SPTs with the following cereal grains and grasses were applied to study patients on the day of admission: cereal grains were wheat, rye, barley, oat, rice, and corn; and grasses were timothy, orchard, and Bermuda (Greer Laboratories, Lenoir, N.C.). A positive SPT response was defined as a wheal 3 mm larger than the negative (saline solution) control. Erythema without sufficiently large wheal size was recorded as a negative response.²⁴

Analysis of serum

Serum samples from all study patients were collected and analyzed for total IgE with the PRIST assay (Pharmacia, Uppsala, Sweden), as described by the manufacturer. Serum samples from the 15 selected study patients were also tested by immunoblot analysis. Serum from one nonatopic individual and an IgE myeloma protein served as controls in documenting the presence or absence of nonspecific binding on immunoblot analyses.

Preparation of cereal grain extracts

Cereal grain and gluten extracts were prepared from the respective flours by mixing 10 gm of flour with 30 ml of phosphate-buffered saline (PBS). Samples were mixed on a rocker for 4 hours at 4° C. Mixtures were centrifuged at 2500 g for 10 minutes, then supernatants were collected and centrifuged at 17,000 g for 10 minutes. Extracts were sterile-filtered, lyophilized, and stored at 4° C. Protein determination of the extracts was performed with a commercially available protein determination assay (BioRad, Inc., Richmond, Calif.).

Preparation of grass extracts

Lyophilized samples of each grass pollen were kindly provided by Greer Laboratories. Each vial was reconstituted with 2 ml of deionized, distilled water. Protein determination was performed on each grass mixture (Pierce Chemical Co., Rockford, Ill.). Samples were then aliquoted and stored at -20° C.

Gel electrophoresis

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed on six cereal grains (wheat, rye, barley, oat, rice, and corn), wheat gluten, and four taxonomically related grasses (timothy, meadow, fescue, Bermuda, and sweet vernal). Cereal grain extracts were reconstituted with PBS to yield the following final concentrations: 0.5 mg/ml for wheat, gluten, and corn; 0.35 mg/ml for rice; 1 mg/ml for rye; and 50 mg/ml for barley and oat. All samples were diluted 5:1 with sample buffer consisting of 0.4 mol/L Tris-HCl, pH 6.8, 8% dithiothreitol, 10% SDS, and 50% glycerol with bromophenol blue and pyronin Y for

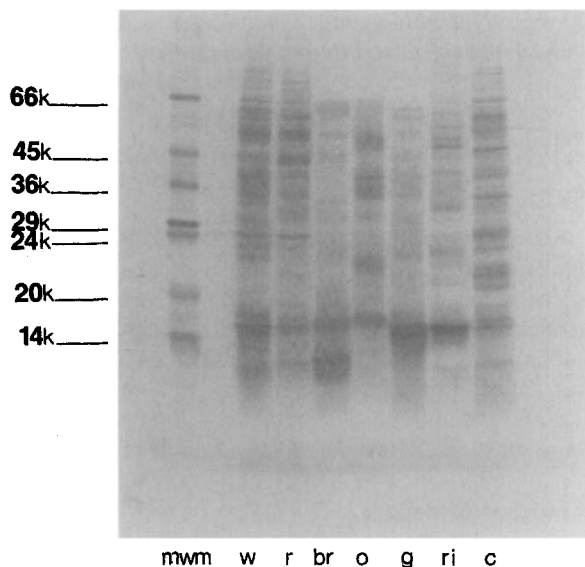


FIG. 2. Amido black staining of the SDS-PAGE sequence for cereal grains. *mwm*, Molecular weight markers; *w*, wheat; *r*, rye; *br*, barley; *o*, oat; *g*, gluten; *ri*, rice; *c*, corn.

tracking. Grass extracts were mixed with PBS to yield a final concentration of 0.25 mg/ml. Samples were diluted 5:1 with sample buffer.

Two different sequences were run, one with cereal grain extracts alone and the other with a combination of cereal grain and grass extracts. Seventy-five micrograms of each cereal grain sample, 50 μ g of each grass sample, and 10 μ g of molecular weight markers (MWs) (Sigma Chemical Co., St. Louis, Mo.) were boiled in sample buffer for 10 minutes and then loaded in the following sequences: cereal grain gels—MWs, wheat, rye, barley, oat, gluten, rice, corn; cereal grain and grass combination gels—MWs, wheat, rye, oat, timothy grass, meadow fescue, Bermuda grass, sweet vernal (Figs. 2 and 3). The proteins were resolved by discontinuous SDS-PAGE, as previously described.¹⁷ The optimal protein concentrations to be loaded on the gels were adjusted to provide clear, sharp bands without excessive diffusion of proteins.

The running gel was prepared from a stock of 33.5% acrylamide and 0.3% N, N'-bis-acrylamide diluted to a final concentration of 13.5%. The stacking gel was prepared from a stock of 30% acrylamide and 0.44% N, N'-bis-acrylamide diluted to a final concentration of 3%. Gels were buffered with Tris-HCl 1 mol/L, pH 9.1, and Tris-HCl 0.5 mol/L, pH 6.8, respectively. Both gels contained 10% SDS and deionized, distilled water and were polymerized with 3% ammonium persulfate and N, N, N', N' tetramethylethylenediamine. The electrophoresis tank buffer was a mixture of 50 mmol/L Tris base, glycine, and SDS diluted with deionized, distilled water. Current was applied at 80 V for 1 hour, then at 160 V for 3 hours to allow proteins to fully separate.

Immunoblotting

The proteins were transferred from the polyacrylamide gels onto nitrocellulose paper by means of electrophoretic transfer overnight at 0.15 A, as previously described.¹⁷ Tank buffer was made with 50 mmol/L Tris and glycine, pH 9.1, and 20% methanol. The initial nitrocellulose blots were stained with 1% amido black to ensure that all proteins had been adequately transferred without diffusion or mixing of protein fractions between lanes. Further nitrocellulose immunoblotting was performed for each patient. Nonspecific binding sites on the nitrocellulose blots were blocked for 2 hours with 0.5% porcine gelatin in PBS, pH 7.4, with 0.05% Tween-20 (PBST gel). Blots were then washed with PBS plus 0.05% Tween-20 (PBST) and probed for antigen-specific IgE binding to the cereal grain and grass protein bands. Nitrocellulose blots were incubated on a rocking platform for 3 hours at room temperature with a 1:25 or 1:50 dilution of patient or control sera in PBST gel. Sera dilutions were optimized to produce maximal band intensity and minimal background signal. Blots were washed for 5 minutes each with 5 exchanges of PBST. The blots were then incubated for 2 hours on a rocking platform with a 1:500 dilution of horseradish peroxidase-labeled rabbit anti-human IgE (Dako Corp., Carpinteria, Calif.) in PBST gel. Washing steps were repeated with 5 exchanges of PBST. The blots were then developed in 10 mmol/L potassium phosphate, pH 6.8, with 10 mmol/L ethylenediaminetetraacetic acid containing 0.009% hydrogen peroxide and 0.3 mg/ml 3,3'-diaminobenzidine. The blots were developed in an environment devoid of light for 3 to 15 minutes until full protein banding was evident. Reactions were then stopped by washing with PBS.

Densitometer readings

Immunoblots were analyzed on a Laser densitometer (Pharmacia LKB Biotechnology, Inc., Piscataway, N.J.). To determine the approximate molecular weight of each grain and grass protein, the distance traveled for each protein was compared with those of known MWs. The molecular weight of each protein fraction was then used to compare antigen binding patterns between patients.

RESULTS

One hundred forty-five of a total of 360 patients (40%) evaluated to date for food hypersensitivity were suspected of having cereal grain hypersensitivity on the basis of SPT results. The median age of study patients was 4.5 years, with a range of 3 months to 30 years. The median IgE concentration of study patients was 3400 IU/ml, with a range of 0 to 88,853 IU/ml. The median IgE concentration for groups 1, 2, and 3 was 5425 IU/ml (range, 241 to 57,800 IU/ml). Medians for individual groups were: group 1, 1375 IU/ml (range, 358 to 57,800 IU/ml), group 2, 3266 IU/ml (range, 241 to 21,200

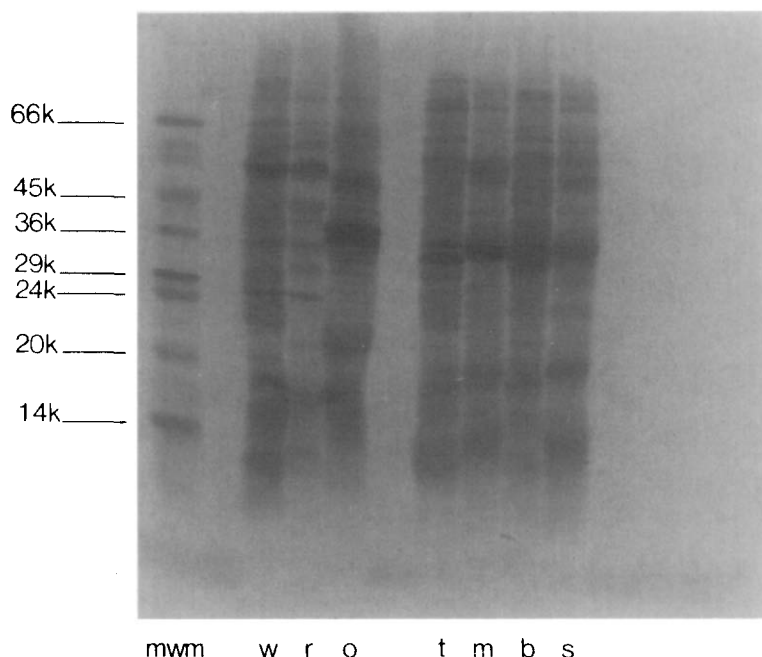


FIG. 3. Amido black staining of the SDS-PAGE sequence for cereal grains and grasses. *mwm*, Molecular weight markers; *w*, wheat; *r*, rye; *o*, oat; *t*, timothy; *m*, meadow fescue; *b*, Bermuda; *s*, sweet vernal.

TABLE I. SPT results in 145 patients

	Total SPTs	Positive SPT results	% Positive
Grains			
Wheat	145	82	57
Rye	113	46	41
Barley	44	20	45
Oat	127	35	28
Rice	98	23	23
Corn	98	24	24
Grasses			
Timothy	84	59	70
Orchard	53	39	74
Bermuda	85	57	67

IU/ml), and group 3, 6765 IU/ml (range, 603 to 48,400 IU/ml). No statistical difference ($p > 0.10$) was found between groups as determined by the Wilcoxon rank sum test. Eighty-nine percent of patients had atopic dermatitis, and 38% had a combination of atopic dermatitis and asthma or allergic rhinitis or both. A positive family history of atopy was elicited from 93% of the patients.

SPT results

The 145 patients evaluated for cereal grain hypersensitivity had 629 SPTs performed with the

TABLE II. Test outcomes in 145 patients

	Patients grain challenged (<i>n</i> = 145)	Patients with positive SPT results (<i>n</i> = 145)	Patients with positive challenge (<i>n</i> = 31)	% False- positive*
Wheat	126	82	26	69
Rye	7	5	4	20
Barley	12	11	4	64
Oat	29	18	5	72
Rice	9	4	1	75
Corn	45	17	5	71

*Represents the percentage of patients with positive SPT results but with no evidence of clinical reactivity as determined by food challenge.

six cereal grains. SPTs with cereal grains were performed on the basis of history and were not performed with all grains in all patients. SPTs with grasses (timothy, orchard, Bermuda) were performed in 84 study patients at the time of challenges. Exclusions were made on the basis of patient age, availability of skin surface, and clinical history (Table I).

Food challenge results

A total of 228 food challenges with suspected cereal grains were performed in 145 patients



FIG. 4. Immunoblot panel probed with IgE myeloma protein.

(Table II). Specific cereal grains studied were chosen on the basis of clinical history and SPT results. Among the 145 patients challenged, 45 positive reactions occurred in 31 patients (21%). All adverse reactions were immediate reactions occurring within 1 to 2 hours of challenge. Twenty-five of the 31 patients (80%) had positive challenge responses to only one grain (19 of 25 to wheat; 3 of 25 to corn; 1 of 25 each to barley, rice, and rye). Three patients (10%) had positive challenge responses to two grains (2 of 3 to wheat and oat, 1 of 3 to wheat and rye). Three patients (10%) had positive challenge responses to four grains (1 of 3 to wheat, oat, barley, and corn; 1 of 3 to wheat, oat, rye, and corn; 1 of 3 to wheat, oat, barley, and rye). Thus only 21% of patients suspected of having cereal grain hypersensitivity were confirmed to be sensitive by challenge, and 80% of patients were reactive to only one cereal grain. The majority of

TABLE III. Comparison of tests in 15 study patients

	Group 1 (n = 4)	Group 2 (n = 5)	Group 3 (n = 6)
Cereal grain DBPCFC (+)	4	5	0
Cereal grain SPT (+)	4	4	5
≥3 SPT (+)	4	4	1
Grass SPT (+)	4	0	6
≥3 SPT (+)	2	0	6

cereal grain reactions were observed in response to wheat: 26 of the 31 patients with cereal grain reactivity (84%) showed wheat hypersensitivity as confirmed by DBPCFC ($n = 25$) or history of anaphylaxis ($n = 1$). Sixty-seven percent of patients with positive SPT responses had no clinical evidence of hypersensitivity when specific food challenges were performed, a substantial false-positive rate when SPTs are compared with food challenges (Table II).

Of the 145 patients evaluated for food hypersensitivity, 15 were selected to study specific antibody sensitivity to cereal grains and grasses. These patients were separated into three groups on the basis of food challenges and SPT results (Table III). Group 1 consisted of four patients with cereal grain and grass allergies. According to oral challenge results, one patient was reactive to wheat and oat; one to wheat and barley; one to wheat, rye, barley, and oat; and one to wheat, rye, barley, and corn. Group 2 consisted of five patients with wheat allergy alone who had no evidence of other grain allergy as determined by DBPCFC or SPT and no evidence of grass allergy according to history or SPT results. Group 3 consisted of six patients who were allergic to grass with positive SPT responses to all three test grasses and negative DBPCFC responses to cereal grains.

SDS-PAGE

Figs. 2 and 3 show amido black staining of the two SDS-PAGE sequences, cereal grains alone, and cereal grains plus grasses. Both figures show extensive protein binding among all lanes. By SDS-PAGE, proteins were separated into numerous fractions ranging from 66 kd to 10 kd: wheat, 32 kd; rye, 26 kd; barley, 27 kd; oat, 20 kd; gluten, 19 kd; rice, 27 kd; corn, 30 kd; timothy, 26 kd; meadow fescue, 17 kd; Bermuda, 20 kd; and sweet vernal, 20 kd.

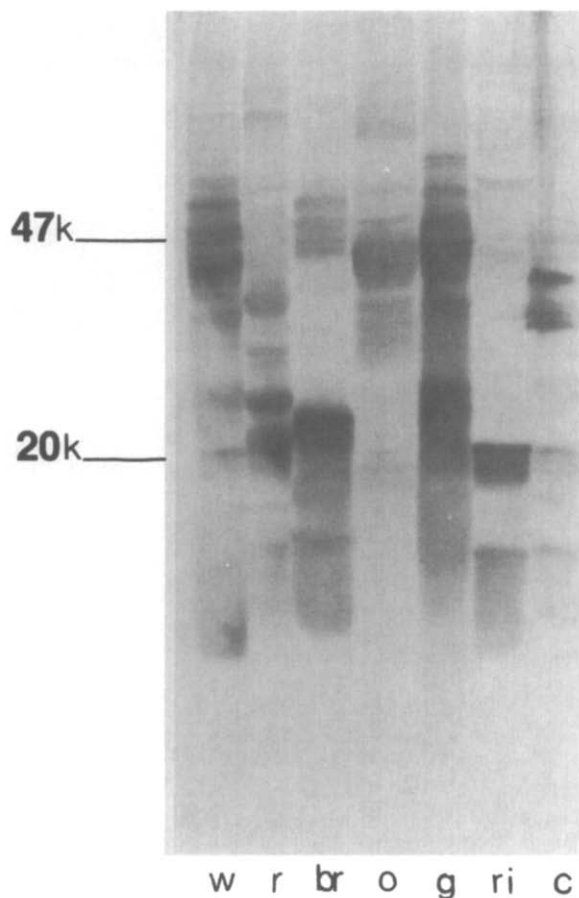


FIG. 5. Immunoblot probed with patient serum from group 1. Numerous cross-reactive bands were identified among grains, despite the fact that this patient was allergic to only two of the grains (wheat and barley).

Immunoblot analyses

To assess the specificity of binding on immunoblot analysis, nonspecific binding was tested by separately probing immunoblot panels with control sera, IgE myeloma protein, and secondary antibody with horseradish peroxidase (Fig. 4). Rabbit anti-human IgE antibody (secondary antibody) was selected after preliminary studies indicated that rabbit antisera had less nonspecific binding than goat antibody preparations. Fig. 4 is an immunoblot panel probed with IgE myeloma protein. Overall, low nonspecific binding occurred among most of the cereal grains and grasses. Similar results were seen on blots probed with control sera and secondary antibody, indicating secondary antibody as the cause of nonspecific binding. Similar results were also seen with cereal grain immunoblots alone (data not shown). The wheat protein lane showed very little binding, only at fractions 14 kd, 18 kd, 26 kd, and 29 kd. Oat protein showed the highest degree of nonspecific binding, which may

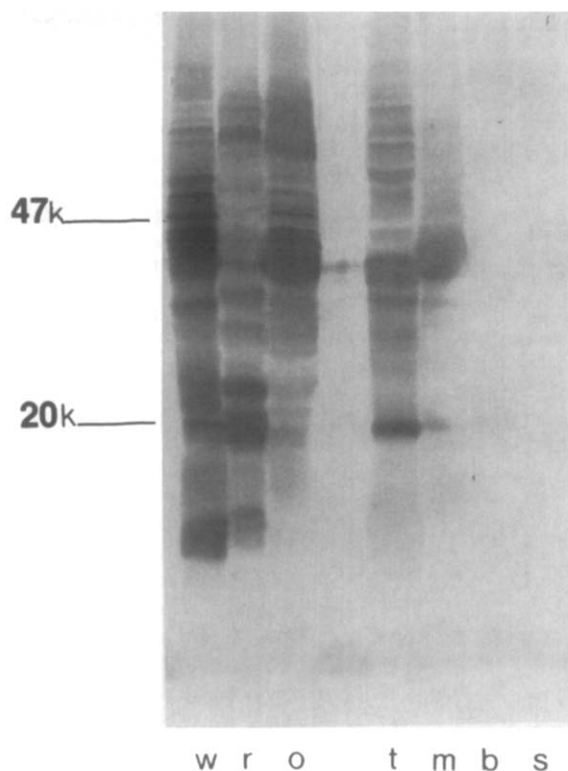


FIG. 6. Immunoblot of grains and grasses probed with serum from the patient in group 1 who was allergic to wheat, barley, and grasses.

be caused by the presence of lectins in cereal grain extracts.

After transfer to nitrocellulose, cereal grain immunoblot panels were probed with sera from patients in group 1 (with grain and grass allergies) to analyze the degree of *in vitro* cross-reactivity among various cereal grains. Patients exhibited extensive cross-reactivity (IgE binding) among the different cereal grains. As represented in Fig. 5, numerous cross-reactive bands were identified among grains, despite the fact that this patient was allergic to only two of the grains (wheat and barley). No significant difference in *in vitro* cross-reactivity was seen between the patients allergic to two grains compared with those allergic to four grains (data not shown). Extensive binding to gluten was seen in all blots as represented in Fig. 5. As shown in Table III, *in vitro* cross-reactivity did not correlate with clinical hypersensitivity in patients with cereal grain allergy.

Combination immunoblots of cereal grains and grasses were probed with individual serum samples from all groups. Serum samples from patients in groups 1 and 2 were used to assess the degree of cross-reactivity observed between cereal grains and between taxonomically related grasses. Serum

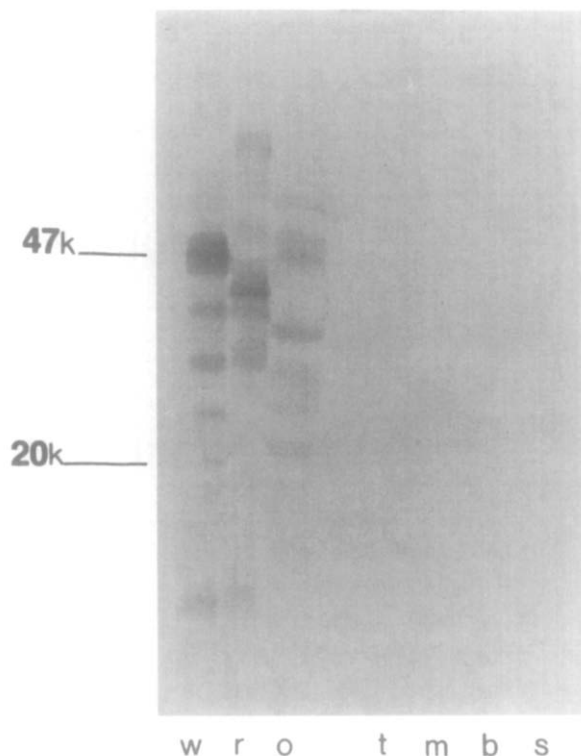


FIG. 7. Representative immunoblot from a patient only allergic to wheat in group 2 who had negative SPT responses to grasses.

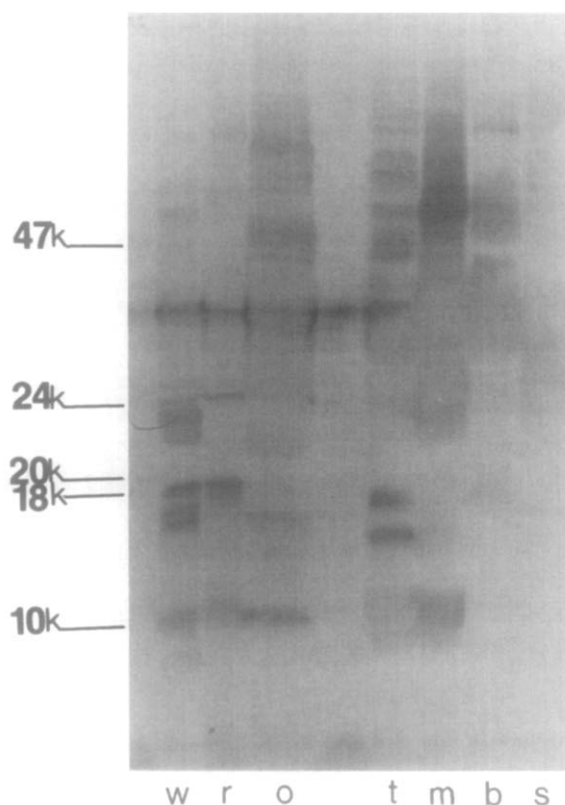


FIG. 8. Representative immunoblot from a patient in group 3 who was allergic to grasses but no cereal grains.

samples from both patient groups showed extensive cross-reactivity among cereal grains, whether reactive to a single grain (wheat alone) or multiple grains. Fig. 6 is an immunoblot from the patient in group 1 who was allergic to wheat, barley, and grasses. As seen in the immunoblot of patient serum in Fig. 5, significant binding occurred with all grains tested. Additionally, two of the four grasses tested also showed extensive binding. The two grasses with extensive binding (timothy and meadow fescue) are members of the same subfamily as wheat (see Fig. 1); however, sweet vernal grass, which showed low cross-reactivity, is also a member of the same subfamily. This emphasizes the lack of clinical relevance of taxonomic relationships and *in vitro* cross-reactivity. Fig. 7 is a representative blot from a patient allergic to wheat in group 2 who had negative SPT responses to grasses. Very dense IgE binding was seen in response to wheat, rye, and oat; and essentially none was seen in response to grasses. As seen in Fig. 8, patients in group 3 (grass allergy alone) displayed extensive IgE antibody binding to various cereal grains and grasses despite the lack of clinical reactivity. In group 3, five of six patients had one or more positive SPT responses to cereal grains. One of the six patients studied showed *in vitro* grain

cross-reactivity by immunoblot analysis yet had no evidence of grain-specific IgE as determined by SPT or food challenge. This observation further indicates the nonspecific nature of *in vitro* testing.

Wheat hypersensitivity accounts for the majority of grain reactivity among patients with food allergies. Therefore further analysis of patients with wheat allergy from groups 1 and 2 was undertaken in an attempt to better characterize IgE antibody specificity in wheat hypersensitivity. Specific IgE binding to a 47 kd and/or 20 kd wheat fraction was seen in eight of nine study patients with wheat allergy. Seventy-five percent of those patients had binding at both 47 kd and 20 kd fractions, and 25% had binding at the 20 kd fraction alone. These fractions were unique to patients with wheat allergy because no patients with grass allergy had IgE antibodies that bound to these fractions (Figs. 5, 6, and 7). Patients with grass allergy had nonspecific binding to several wheat protein fractions (e.g., 24 kd, 18 kd, and 10 kd) (Fig. 8). This nonspecific binding may account for the high apparent IgE cereal grain reactivity observed in RASTs performed for patients with grass allergy.^{9,11} Analysis of other cereal grains was not performed because of a much

lower incidence of clinical reactivity and a restricted patient population.

DISCUSSION

By examining a referral population of children with atopic dermatitis, this study has shown that clinically relevant cross-reactivity among cereal grains is more common than that found in other food families (i.e., legumes). About 20% of patients were clinically reactive to more than one cereal grain. By comparing DBPCFCs with *in vivo* and *in vitro* testing, such as SPTs and immunoblot analyses of sera, our study indicates a very high degree of cross-reactivity in laboratory studies and emphasizes the need for controlled challenges in diagnosing food hypersensitivity. Hoffman and Haddad⁹ previously reported that RAST analysis was useful in diagnosing food allergy. They studied 127 children with suspected food hypersensitivity as determined by history and reported 75% with one or more positive food-specific RAST results, but they did not verify the clinical relevance of these test results with oral challenge studies. Wraith et al.¹¹ also reported that RASTs were useful in evaluating patients with suspected food hypersensitivity, claiming 79% sensitivity and 97% specificity when RASTs were compared with elimination diets. Wraith et al.¹¹ did state, however, that RAST positivity did not confirm clinical reactivity. Several investigators have shown that history is an unreliable tool in diagnosing food hypersensitivity and that SPTs and RASTs frequently show clinically insignificant positive results, making all three poor predictors of food hypersensitivity.²⁵⁻²⁸

Much confusion has arisen regarding food hypersensitivity to cereal grains from previous studies of patients with baker's asthma. Baker's asthma is the result of a type I, IgE-mediated antibody response to inhaled flour. This disease entity has been extensively studied, with wheat being the primary allergen of exposure. Bjorksten et al.¹² reported an 85% correlation between SPT and RAST results in patients with clinically proven baker's asthma caused by wheat. Many investigators have demonstrated a good correlation between disease and the presence of wheat-specific antibody in sera.^{3, 5, 12} Sutton et al.⁴ further reported increased wheat-specific IgE in sera from children with suspected food hypersensitivity. They subsequently recommended that "wheat flour sensitive patients should be warned to avoid cereal bran in their diet." This recommendation was based on the assumption that RAST positivity indicated clinical reactivity. They further recommended elimi-

nation diets to confirm the diagnosis but reported no controlled food challenges or elimination diets in their study. On the basis of our observations and data collected, there is no evidence that sensitization caused by inhaled cereal grains imparts sensitization to ingested cereal grains.

Although both disease entities arise through IgE-mediated mechanisms, the mode of sensitization is quite different, thus physiologically separating the disease processes.

Armentia et al.²⁹ have reported successful immunotherapy with commercial wheat flour extracts in treating 16 patients with baker's asthma. They reported success in lowering bronchial reactivity, skin reactivity, and specific IgE levels after 10 and 20 months of therapy.²⁹ Such success has not been possible with food hypersensitivity to date. A recent study by Oppenheimer et al.³⁰ reported promising preliminary results with a rush immunotherapy protocol for peanut desensitization. However, the study was prematurely halted by an accidental death, underscoring the potential dangers in use of current immunotherapy techniques for food hypersensitivity.

Baldo et al.³ have further studied the various wheat protein fractions in prototypic diseases of hypersensitivity. They found four major wheat protein fractions, two water-soluble fractions (albumin and globulin) and two water-insoluble fractions (glutenin and gliadin). Using RAST scores and SDS-PAGE of the various fractions, Baldo et al.³ have shown that different fractions are important in different disease entities. Albumin was found to be the primary fraction of importance in baker's asthma, globulin in food hypersensitivity, and glutenin in celiac disease.^{3, 4} By immunoblot analysis, Sutton et al.⁴ showed that sera from patients with baker's asthma displayed IgE binding to many flour proteins with individual variations. Likewise, in this study we have shown extensive IgE binding among many flour proteins, also with individual variations.

Further analysis of the water-soluble components of wheat flour was performed by Pfeil et al.³¹ They evaluated patients with hypersensitivity to inhaled flour allergens (i.e., baker's asthma), using SDS-PAGE and immunoblot analyses. By using a panel of four monoclonal antibodies, they identified three major allergens with molecular weights of 47, 17, and 15 kd. These fractions were identified by their ability to bind IgE to 50% of allergic sera.³¹ In our food-hypersensitive patients with wheat allergy, we found two major allergens at 47 kd and 20 kd. These bands were recognized by sera

of 89% of patients allergic to wheat, as diagnosed by DBPCFC. Sera of patients with grass allergy did not recognize either band but did show nonspecific binding at 24, 18, and 10 kd. These unique binding sites may prove to be useful in differentiating patients with wheat allergy from those with grass allergy by means of in vitro tests.

In evaluating patients with cereal grain hypersensitivity, both by inhalation and ingestion, many groups of investigators have noted the strong in vitro cross-reactivity with grass allergens.* These authors report positive SPT and RAST responses to taxonomically related grasses in patients with grain sensitivity. Our study also shows extensive cross-reactivity among cereal grains and grasses with SPTs and immunoblot analyses in patients who have both grain and grass allergies or who have grass allergy alone. Our findings differ, however, in the group of patients with wheat allergy alone. When grain and grass combination immunoblots were probed with sera of patients with wheat allergy, extensive cross-reactivity was seen among cereal grains but none among taxonomically related grasses (Fig. 7). Four other members of this particular study group had similar results (data not shown). Interestingly, when similar blots were probed with grass allergen sera, extensive cross-reactivity was seen among individual grains and grasses, as well as between grains and grasses. There is no obvious explanation for these findings; however, they emphasize the lack of specificity of in vitro testing in the diagnosis of cereal grain hypersensitivity.

In summary, only 21% of patients suspected of having cereal grain hypersensitivity, as determined by history or SPT results, had symptomatic reactivity as determined by DBPCFC, and 80% of reactions occurred in response to only one cereal grain (76% to wheat). Patients with wheat allergy alone showed extensive in vitro cross-reactivity to other grains but little to taxonomically related grasses, whereas patients with grass allergy alone showed extensive in vitro cross-reactivity to both cereal grains and grasses. This observation indicates that the elimination of all grains from the diet of a patient with grain allergy is clinically and nutritionally unwarranted. Controlled elimination diets with food challenges remain the gold standard in the diagnosis of food hypersensitivity. However, further purification and characterization of wheat fractions 47 kd and 20 kd may provide more sensitive and specific in vitro testing.

*References 1, 4, 9, 11, 13-15, and 29.

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