
Case report

Anaphylaxis to lemon soap: citrus seed and peanut allergen cross-reactivity

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Background: Many individuals allergic to peanuts have multiple allergen sensitivity.

Objective: To report the first case, to our knowledge, of a peanut allergic patient who exhibited cosensitivity to citrus seeds and who had experienced anaphylaxis to lemon soap.

Methods: Extracts of peanut and seeds from different varieties of citrus fruit (orange, lemon, and mandarin) were prepared and resolved with 14% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Direct and inhibition immunoblotting of the patient’s serum on the extracts was used to examine the pattern of IgE reactivity and the presence of cross-reactive allergens.

Results: Numerous IgE reactive proteins were demonstrated in each citrus seed extract and the peanut extract. Complete IgE cross-reactivity was demonstrated among the different citrus seed extracts. Partial cross-reactivity was demonstrated between the peanut and orange seed extracts.

Conclusions: Citrus seeds contain numerous IgE reactive proteins that are completely cross-reactive among orange, lemon, and mandarin. When peanut allergy coexists with citrus seed allergy, IgE cross-reactivity between peanut and citrus seed proteins can be demonstrated, suggesting a basis to this cosensitivity.

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INTRODUCTION

Peanut allergy is present in 1% to 2% of the general population and is the major cause of life-threatening or fatal food-induced anaphylaxis.¹ Up to 40% of peanut allergic patients show cosensitivity to tree nuts, and peanut and/or tree nut allergic patients often share sensitivity to other foods, including sesame seeds, poppy seeds, coconut, stone fruit, legumes, and lupine.² To date, only limited data exist with regard to hypersensitivity to citrus fruit,³ and allergenic components of citrus extracts are not fully defined. The major protein component of citrus seeds is the globulin seed storage protein citrin,⁴ but whether citrins cross-react between different citrus fruits or with similar seed storage proteins in peanut is not known. In this study, we report the first case, to our knowledge, of a patient who shared sensitivity to citrus seeds, peanuts, and tree nuts and demonstrate cross-reactivity among different citrus fruit seed proteins and between orange seed and peanut via inhibition immunoblotting experiments.

METHODS

Case Report

A 26-year-old man with a history of mild asthma presented to the outpatient clinic having recently experienced a documented episode of anaphylaxis after showering and washing his torso and face with lemon-impregnated soap. He reported experiencing anaphylaxis previously on ingestion of whole crushed orange juice and citrus seeds, including lemon, orange, and mandarin seeds, but not processed citrus fruit juices. Such reactions occurred in a stereotypical fashion, producing laryngeal edema, generalized urticaria, and asthma symptoms within minutes of ingestion. He experienced similar symptoms after ingestion of peanut and tree nuts, including hazelnut, Brazil nut, macadamia nut, pine nut, and almond.

Skin prick testing against a standard panel of aeroallergens revealed the patient to be nonatopic. Serum IgE antibodies against peanut, orange, lemon, walnut, and hazelnut were demonstrated (4.0, 4.0, 1.1, 15.2, and 6.77 kU_A/L, respectively; Pharmacia CAP System, Pharmacia Diagnostics, Uppsala, Sweden). The patient was advised to avoid all tree nuts, peanut, and citrus fruits and prescribed an autoinjectable adrenaline pen. The study was approved by the Alfred Hospital Ethics Committee, and informed consent was obtained.

Preparation of Extracts

Peanut extract was prepared as described previously⁵ and dialyzed against phosphate-buffered saline (PBS) at 4°C, using a 3.5-kDa cutoff dialysis membrane (Pierce, Rockford,

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IL). Extracts were prepared from orange (*Citrus sinensis* cultivar Valencia), mandarin (*Citrus reticulata* cultivar Kara), and lemon (*Citrus limon* cultivar Lisbon) seeds similarly.

SDS-PAGE and Immunoblotting

Peanut and citrus seed extracts were resolved with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using 14% gels under reducing conditions as described by de Leon et al,⁵ and proteins were stained with Coomassie brilliant blue (Sigma, St Louis, MO). For immunoblotting, proteins separated by 14% SDS-PAGE were transferred onto nitrocellulose membranes as outlined previously.⁵ Membranes were blocked in 10% milk powder–PBS then incubated overnight with the patient or nonpeanut allergic, atopic control serum diluted 1:5 with 1% milk powder–PBS–0.05% Tween. After washing, the membranes were incubated with rabbit anti-human IgE horseradish peroxidase conjugate (2.6 µg/mL in 1% milk powder–PBS–0.05% Tween; Dako, Carpinteria, CA) for 1 to 2 hours at room temperature. After washing with PBS–0.05% Tween, membranes were incubated for 1 minute with chemiluminescence substrate (Du Pont, Wilmington, DE) then analyzed using Labworks image acquisition software (UVP Laboratory Products, Cambridge, England).

Inhibition Immunoblotting

Serum samples were diluted 1:5 with 1% milk powder–PBS–0.05% Tween and preincubated with antigen extract (25 to 100 µg/mL of serum) for 2 hours before immunoblotting as described herein. To exclude reduction in IgE binding due to proteases present in extracts, complete protease inhibitor cocktail tablets were added to the diluent (Roche, Mannheim, Germany).

RESULTS

Characterization of Citrus Seed and Peanut Extracts

Analysis of the peanut and citrus seed extracts by SDS-PAGE is illustrated in Figure 1. Citrus seed extracts displayed similar profiles, indicative of close phylogenetic relationships. Protein bands were visible between 9 and 61 kDa for each extract, with strong bands at 9, 14, 15, and 27 kDa. Bands present between 9 and 15 kDa in all 3 extracts may represent the pan-allergens profilin and lipid transfer protein.⁶ The faint band at 51 kDa may represent the intact citrin protein, and the bands at 22 and 33 kDa may represent citrin subunits.⁴ Albumin seed storage proteins have also been described for citrus seeds,⁴ with prominent bands at 40 and 42 kDa on SDS-PAGE; bands in this region are only faintly visible in our extract, probably because of differences in protein extraction techniques. Multiple protein bands in the citrus seed extracts could be seen at similar molecular masses to those in the peanut extract, but whether these represent homologous proteins is unclear in the absence of further characterization.

IgE Immunoblotting of Citrus Seed and Peanut Extracts

The patient's serum IgE bound to orange seed extract proteins, with molecular masses ranging from 9 to 48 kDa (Fig

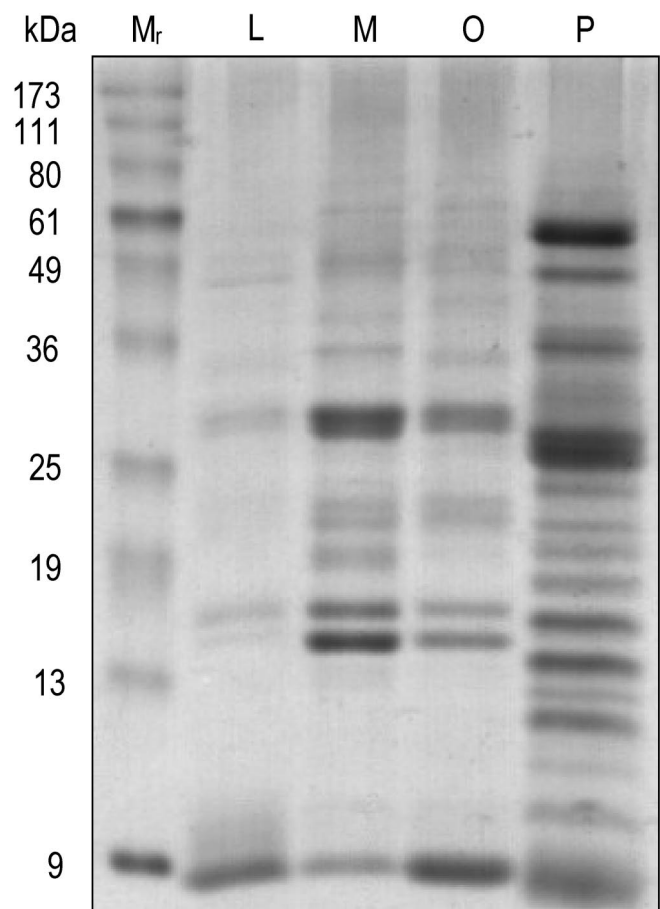


Figure 1. Analysis of citrus seed and peanut extracts. Orange (O), lemon (L), and mandarin (M) seed extracts were resolved under reducing conditions with 14% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue. M_r indicates molecular mass markers.

2). Several bands were recognized, with the most intense at 9, 22, 27, 42, and 48 kDa. For the peanut extract, patient serum IgE bound to protein bands, representing the major allergens Ara h 1, Ara h 2 and Ara h 3, along with other bands of unknown identity. No IgE binding to either extract was observed with the non-peanut allergic, atopic control serum (data not shown).

Inhibition Immunoblotting

Preincubation of the patient's serum with the orange, mandarin, or lemon seed extract led to near total abrogation of IgE binding to all allergenic proteins within the orange seed extract (Fig 3). The secondary antibody bound nonspecifically to a 9-kDa protein, but this signal was markedly lower compared with the strips incubated with serum alone. Preincubation with peanut extract led to loss of signal from all protein bands except those at 27 and 9 kDa. This finding suggests that complete IgE cross-reactivity occurs for proteins contained within the orange, lemon, and mandarin seed

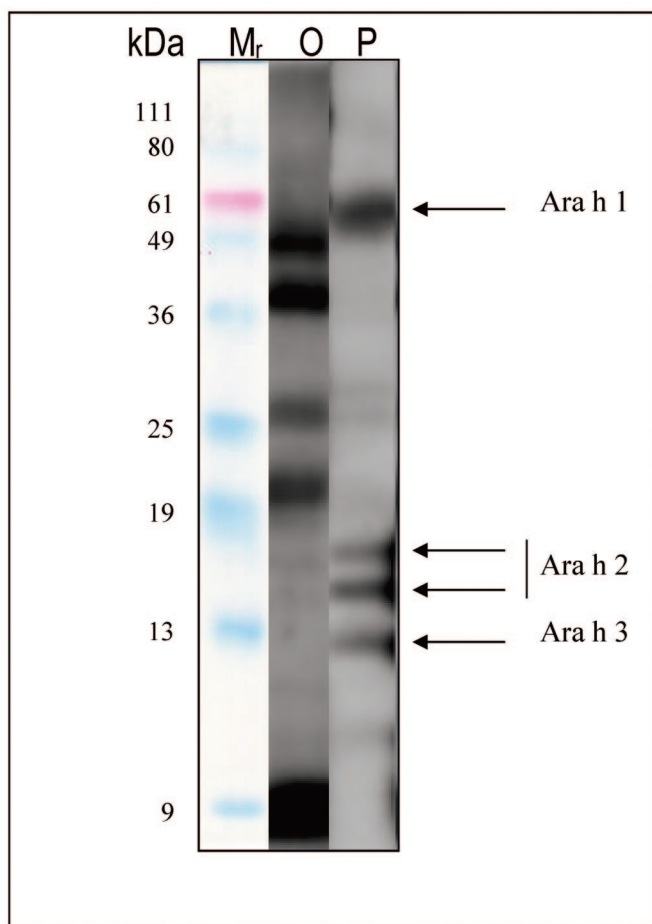


Figure 2. Immunoblotting of peanut (P) and orange seed (O) extract. The P and O extracts were resolved with 14% sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reducing conditions then transferred to nitrocellulose and probed with the patient serum at a 1:5 dilution. M_r indicates molecular mass markers.

extracts, but only partial IgE cross-reactivity is present between orange seed and peanut. Nonspecific inhibition of IgE binding by the peanut extract was excluded by preincubation of major natural rubber latex allergen Hev b 5 specific IgE with the peanut extract before immunoblotting on recombinant Hev b 5 (data not shown). The control protein, keyhole limpet hemocyanin, failed to inhibit at any concentration.

DISCUSSION

Allergy to citrus fruits, most notably orange, is well documented, but the allergenic proteins have only been identified recently. Lipid transfer proteins and profilins, both widely recognized as pan-allergens in many different plant foods, contribute to the allergenicity of oranges.^{7–9} Germin-like proteins in orange are also described as allergenic,⁸ although in vivo reactivity is reported to be low.¹⁰ Only limited data on citrus seed allergens exist, with a single study from China demonstrating allergenicity of seeds on the basis of skin

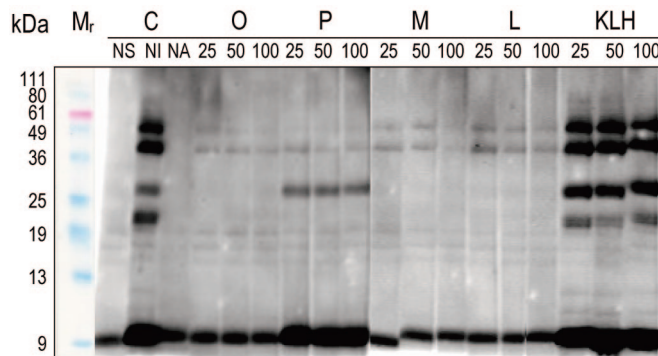


Figure 3. Inhibition immunoblotting of orange seed (O) extract. The O extract was resolved with 14% sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reducing conditions then transferred to nitrocellulose and probed with the patient serum. Serum was preincubated with lemon seed (L) or mandarin seed (M) extract, peanut (P) extract, or O and keyhole limpet hemocyanin (KLH) extracts as positive and negative controls, respectively. M_r indicates molecular mass markers; C, control strips: NS, no serum; NI, no inhibitor; NA, nonallergic atopic serum.

testing with individual components of orange.³ The present study, therefore, provides valuable information on allergenic proteins within citrus seeds. Numerous proteins were visualized for the orange seed extract, but only 5 of these showed strong IgE binding, namely, the 9-, 22-, 27-, 42-, and 48-kDa proteins. On the basis of size, some of these allergenic proteins may represent a citrin subunit (22 kDa) and citrus seed albumin (42 kDa).⁴ IgE binding to the 42- and 48-kDa proteins did not correlate with the relative abundance of these proteins within the extract, because they were only poorly visualized with Coomassie brilliant blue staining. Whether the IgE-reactive proteins identified in this study represent major allergens will only become clear from a population study.

Shared sensitivity to citrus seed and peanut is an unusual clinical phenomenon. For this subject, the pattern of serum IgE reactivity to peanut proteins by immunoblotting did not differ from that for other peanut allergic patients,⁵ with similar intensity of IgE binding and number of proteins recognized. This finding suggests that it is the citrus seed reactive antibodies that have led to the cross-reactive immune response. Alternatively, at the molecular level, serum IgE antibodies from this patient may recognize different peanut allergen epitopes that are cross-reactive with citrus seed allergens but are not recognized by most peanut allergic patients.

All 5 IgE binding proteins within the orange seed extract were demonstrated to be cross-reactive with the other citrus seeds tested, a not unexpected finding given their phylogenetic similarities. Surprisingly, the unrelated peanut showed cross-reactivity with 3 of the 5 IgE-binding orange seed proteins (22, 42, and 48 kDa). Based on the molecular mass, the 22-kDa IgE-binding protein in orange seed may represent citrin, which shows amino acid homology with the peanut

allergens Ara h 3 and Ara h 4 (49%) and with the major peanut allergen Ara h 1. Ara h 3 and Ara h 4 are members of the glycinin seed storage protein family, whereas Ara h 1 is classified as a vicilin seed storage protein. Another peanut allergen, Ara h 8, is classified as a profilin,¹¹ which is a known plant pan-allergen. Thus, seed storage proteins and profilin may be the likely sources of cross-reactivity between citrus seeds and peanut. In this case study, the putative 9-kDa lipid transfer protein of orange seed extract showed strong IgE cross-reactivity with other citrus seeds consistent with its status as a pan-allergen¹² but did not cross-react with peanut.

Whether IgE reactivity with each of these proteins is clinically relevant or in fact represents clinically irrelevant cross-reactivity as may occur with carbohydrate epitopes^{13,14} is unclear. Whole crushed fruit juices may contain citrus seed extracts, although most drinks are processed and do not contain pulp. Conceptually, unintentional ingestion may occur in restaurants; hence, patient education is important.

CONCLUSION

We demonstrated that a citrus seed sensitized patient has serum IgE reactive to numerous proteins within orange seed extract that ranged in size from 9 to 48 kDa. IgE-reactive orange seed proteins were completely cross-reactive with lemon and mandarin seed proteins, and as such, patients sensitized to one citrus fruit should be advised to avoid all citrus fruits. When a patient shows cosensitivity to peanut and citrus seeds, as in this case, cross-reactivity can occur for multiple proteins, likely because of the presence of pan-allergens within each protein but probably not via lipid transfer protein.

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