

Latex allergy can induce clinical reactions to specific foods

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Summary

Objective The purpose of this study was to investigate crossreactivity between latex and foods, to identify crossreacting IgE binding proteins, and to assess the clinical significance.

Methods Forty-seven latex allergic patients and 46 non-latex allergic patient controls were studied. Allergen sensitization was determined by skin-prick testing (SPT) and allergenic proteins were identified by immunoblot reactivity and amino acid sequence analysis.

Results Immunological reactivity to foods was found to be common, occurring in 33 latex-allergic individuals but in only seven controls ($P < 0.000001$); 100 of 376 (27%) food skin-prick tests were positive in the latex-allergic subjects. Twenty-seven out of 100 positive food SPTs were associated with clinical symptoms. Seventeen patients manifested a clinical allergy to at least one food including 11 with anaphylaxis, and 14 with local sensitivity reactions. Positive food skin tests occurred most frequently with avocado (53%), potato (40%), banana (38%), tomato (28%), chestnut (28%), and kiwi (17%). Latex-allergic patients (23%) recognize a protein that had sequence homology to a broad class of plant proteins known as patatins. Crossreactivity between latex and several potato proteins was observed by immunoblot inhibition analysis.

Conclusions Sensitization to latex has extensive crossreactivity with certain foods and leads to clinical allergic reactions. Potatoes and tomatoes are newly reported crossreacting foods. Plant proteins with structural homology to latex proteins may predispose to food allergy.

Keywords: latex allergy, latex proteins, patatin, food allergy, crossreactivity, potato allergy

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Introduction

Hypersensitivity to latex is a recognized occupational hazard for individuals exposed to latex [1]. Latex proteins from gloves are easily transferred to the wearer and other individuals [2]. Starch powders on latex gloves adsorb protein and become airborne allergens [3]. Prolonged exposure to these proteins can result in sensitization involving specific IgE antibodies and subsequently the development of clinical latex allergy.

The responsible allergens in natural rubber latex have not been fully identified; however, multiple protein allergens are

involved. Rubber elongation factor, an enzyme thought to be important in the biosynthesis of rubber, has been shown to be one of the allergens [4]. Other latex proteins including hevein preprotein and hevamine have also identified as allergens [5]. The clinical manifestations in individual patients are dependent on the route of exposure [6]. IgE-mediated latex allergy can present as contact urticaria, allergic rhinoconjunctivitis, asthma, and even as life-threatening anaphylactic shock [1].

Latex allergy has been reported to be associated with allergy to certain foods such as banana, avocado, kiwi, peach and chestnuts [7–12] but these were all case reports or case series without comparison subjects. Blanco *et al.* [13] recently reported a series of 25 latex-sensitive subjects and examined controls but did not perform statistical

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comparisons between the groups and did not give the proportion of their 'healthy controls' who were atopic. We report here crossreactivity to food in the largest series of latex-allergic patients, including reactivity to some previously unreported foods, and specifically identify one protein with potential common epitopes.

Materials and methods

Patient population

The research protocol was approved by the Ethics Review Committee of the Ontario Allergy Society. Forty-seven otherwise healthy patients were diagnosed as latex allergic, with a positive history and latex skin-prick test (Table 1). Atopy was defined by a positive personal history of atopy and documentation of at least two positive skin tests to a panel of common inhalant allergens including birch, timothy grass, ragweed, *Alternaria*, *Cladosporium* and cat. Eighty-seven per cent (41/47) of the latex-allergic patients were healthcare workers. The control population was 46 non-latex allergic consenting adults who presented consecutively to an outpatient allergy clinic. Atopic non-latex allergic controls consisted of 25 patients with no history of latex exposure who had allergic rhinoconjunctivitis. The 21 additional controls were not atopic and included seven healthcare workers who had regular latex exposure with eczematous hand dermatitis and 14 individuals with unrelated diagnoses. Food allergy patients were excluded as controls.

Skin testing

Skin-prick tests (SPT) were done on the volar aspect of the forearm using a drop of test solution and the epidermis beneath the drop was pricked with a 25 gauge needle. Skin-prick test sites were wiped clean after 15 min and the weal-and-flare reactions carefully measured. Positive weal-and-flare reactions (at least 5 mm greater than the control weal) were outlined, transferred onto transparent tape and recorded as a permanent record. Allergenic components used for SPT included a standard latex skin-test reagent (Bencard, Mississauga, Ontario, Canada), and eight commercially available food allergen extracts: kiwi (1/10 dilution, Bencard Laboratory); banana, avocado, pineapple, tomato, and potato (1/10 dilution), chestnut and milk (1/20 dilution) (Miles Inc. Elkhart, IN, USA). Histamine (1/1000 dilution, Bencard Laboratory) and the diluent (human serum albumin, 0.3 mg/mL in 0.9% saline, Omega Laboratory, Montreal, Quebec, Canada) were used as the positive and negative control testing reagents, respectively. These food allergens were chosen because avocado, banana, kiwi, chestnut and milk were previously reported to be associated with latex allergy. In addition, several of our patients complained of possible allergy to potato and tomato.

Latex protein

Non-ammoniated latex proteins (NAL) were obtained from the Rubber Research Institute of Malaysia. The proteins were prepared by centrifugation of fresh latex without ammonification and consisted of a mixture of B (middle)

Table 1. Demographic characteristics of latex-allergic patients and controls

Characteristic	Latex-allergic patients (n = 47)	Controls (n = 46)
Atopic/non-atopic	39/8	25/21
Age, mean (range)	34 (10–64)	41 (18–67)
Gender (M/F)	4/43	11/35
*Number of HCWs	41	7 (with non-specific hand dermatitis only)
Clinical allergic symptoms	To latex: ● 44 (98%) Local latex allergy, contact urticaria, eczema. ● 42 (89%) Inhalant latex allergy, rhinoconjunctivitis. ● 20 (43%) Anaphylaxis**	● No history of latex reactions ● Allergic rhinitis in atopics

*HCWs. Includes laboratory, hospital, and dental health care workers. **Anaphylaxis was defined as patients presenting with multi-system involvement including at least two of the following skin (urticaria, angioedema), respiratory (shortness of breath and wheezing), or cardiovascular (lightheadedness and loss of consciousness).

and C (bottom) fractions of the centrifuged latex [5]. The lyophilized NAL protein was reconstituted at 10 mg/mL in phosphate buffered saline (PBS) [5].

Potato extract

Approximately 10 g of fresh potato including a shoot, skin, and attached flesh was diced into small pieces and extracted into phosphate buffered saline with sonication. Large pieces and debris were removed by filtering through a wire mesh screen and particulates were removed by centrifugation. Protein was precipitated with 4 volumes of ice-cold acetone and pelleted by centrifugation. The pellet was recovered by centrifugation, air dried and reconstituted in H₂O. Proteins were isolated from beef muscle by a similar protocol as a control extract.

Cyanogen bromide digestion of P46

Non-ammoniated latex proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 15% polyacrylamide gels as previously described [5]. A band corresponding to the migration of the 46 kDa allergen was cut from the gel and electroeluted using the BioRad apparatus model 422. The electroeluted protein was collected, dialysed against H₂O and lyophilized. The 46 kDa protein was then digested with cyanogen bromide to cleave the protein at methionine residues [14]. The eluted protein was dissolved in 70% formic acid and reacted at room temperature with cyanogen bromide using a 2:1 (w/w) ratio. Following an overnight incubation, the reaction was stopped by diluting with H₂O, then the sample was lyophilized and reconstituted in the original volume.

Protein sequencing

For amino acid sequence analysis, the intact 46 kDa and the cyanogen bromide fragments of 46 kDa protein were separated by SDS-PAGE on 15% gels and transferred to PVDF membrane (Immobilon, Millipore Corp., Marlborough, MA, USA). The protein bands were visualized by staining with Coomassie blue and excised for protein sequencing. Protein sequencing was carried out using a Porton 2090 gas phase microsequencer (Beckman Instruments, Fullerton, CA, USA).

Immunoblot inhibition

Latex specific protein antigens were detected by Western blot using a rabbit antiserum as previously described [5]. The rabbit antiserum was previously demonstrated to recognize the same protein antigens as specific IgE in pooled sera

($n = 20$) of latex allergic patients [5]. We used rabbit sera because the anti-latex titre was significantly higher than the anti-latex IgE levels of allergic patients, making it easier to detect crossreactivity. To assay for crossreacting antigens, a 1/7500 dilution (5 mL) of rabbit anti-latex sera was pre-treated for 1 h with allergen extracts prior to addition to the nitrocellulose membrane. The extracts analysed for immunoblot inhibition included potato (100 μ L), beef (100 μ L) as a non-plant negative control, and ammoniated latex protein (100 μ g) as a positive control. Non-ammoniated latex proteins were separated by SDS-PAGE on 15% polyacrylamide gels and transferred to a nitrocellulose membrane. The nitrocellulose membrane was blocked for 1 h with 2% bovine serum albumin and reacted overnight with the rabbit sera. Following washing with 0.1% Tween-20 in PBS, the blot was reacted for 1 h with a 1/5000 dilution of alkaline phosphatase labelled goat anti-rabbit IgE (Promega, Madison, WI, USA). Visible bands indicating antigenic proteins were produced by developing in the alkaline phosphatase substrate NBT/BCIP (Promega). Failure to visualize a specific band would indicate the presence of a crossreacting antigen.

Statistical methods

The proportions of latex allergic and control subjects reacting to foods were compared using the Mantel-Haenszel chi-square test, with stratification by atopic status.

Results

Immunological food sensitization in latex allergic patients occurred commonly with 33/47 (70%) reacting with a positive SPT to at least one food, while only 7/46 (15%) of control patients had a positive food SPT ($P < 0.000001$). The proportion of latex-allergic patients who were skin-test positive was significantly greater than that among control subjects, for all foods tested except pineapple and milk (Table 2). Positive SPTs to avocado (25/47; 53%), potato (19/47; 40%), and banana (18/47; 36%) occurred most frequently, with avocado being the most commonly recognized food allergen. Latex allergic patients reacted to potato (19/47; 40%) as often as banana (18/47; 38%). Many latex allergic patients also reacted to chestnut and tomato (Table 2). No serious adverse reactions to any SPT reagents were observed.

In total, there were 100/376 (27%) positive food skin tests observed among 33 latex allergic patients. Twenty-seven of these positive tests among 17 patients were associated with clinical symptoms. The prevalence of positive SPTs and clinical reactions to foods among the latex-allergic patients are shown in Table 3. Eleven patients presented with mild local pruritus of the mouth or hands following contact with

Table 2. Proportion of latex-allergic patients and control subjects testing positive to various foods

Food	Latex patients (<i>n</i> = 47)	Control subjects (<i>n</i> = 46)	<i>P</i> -value*
	% of total reacting (% of 39 atopics/% of eight non-atopics)	% of total reacting (% of 25 atopics/% of 21 non-atopics)	
Avocado	53 (54/50)	7 (8/5)	<0.0001
Potato	40 (44/25)	2 (4/0)	<0.0005
Banana	38 (36/50)	0 (0/0)	<0.0001
Tomato	28 (28/25)	2 (4/0)	<0.01
Chestnut	28 (31/13)	4 (8/0)	<0.05
Kiwi	17 (13/38)	0 (0/0)	<0.01
Pineapple	4 (5/0)	4 (4/5)	NS""
Milk	4 (5/0)	2 (4/0)	NS

*Mantel-Haenszel chi-square with stratification by atopic status. ""NS. Not significant.

the specific food. Potato was the allergen most commonly associated with this local sensitivity with reactivity in 7/19 patients with positive potato SPT. Banana was the food most commonly implicated in anaphylaxis. Anaphylaxis occurred in 5/18 patients with a positive banana SPT. Other foods causing anaphylaxis included avocado (four patients), kiwi and tomato (one patient each). Fourteen of 47 (30%) latex allergic patients were skin-test negative to all tested foods.

We have previously reported that 9/40 (23%) of latex allergic healthcare workers recognized a 46 kDa protein found in non-ammoniated latex [5]. This protein was iso-

lated from polyacrylamide gels and digested with cyanogen bromide to cleave the protein at the methionine residues. Cyanogen bromide (CNBr) digestion produced several polypeptide fragments of p46 including 16, 30 and 40 kDa fragments. The 30 kDa fragment was the most abundant. The intact 46 kDa protein and the 30 kDa cyanogen bromide fragment were analysed for primary amino acid sequence (Fig. 1). The N-terminal amino acid sequence of the 46 kDa protein had sequence homology (60%) with patatin, a protein from potato. This homology was to a region beginning 19 amino acids from the patatin N-terminus. Likewise, the 30 kDa cyanogen bromide fragment had sequence

Table 3. Prevalence of positive SPTs and clinical reactions to foods among latex-allergic patients

Food	Total number of positive SPTs	Anaphylaxis	*Local sensitivity	Other	Asymptomatic sensitivity
Avocado	25/47 (53%)	4/25 (16%)	2/25 (8%)	Generalized urticaria 1/25 (4%)	18/25 (72%)
Potato	19/47 (40%)	0	7/19 (37%)	0	12/19 (63%)
Banana	18/47 (38%)	5/18 (28%)	3/18 (17%)	Severe abdominal cramping & nausea 1/18 (5.5%)	9/18 (50%)
Tomato	13/47 (28%)	1/13 (8%)	1/13 (15%)	0	11/13 (85%)
Chestnut	13/47 (28%)	0	1/13 (8%)	0	12/13 (92%)
Kiwi	8/47 (17%)	1/8 (12.5%)	0	0	7/8 (88%)
Pineapple	2/47 (4%)	0	0	0	2/2 100%
Milk	2/47 (4%)	0	0	0	2/2 (100%)
Potato	19/47 (40%)	0	7/19 (37%)	0	12/19 (63%)
Total	100/376 (27%)	11/100 (11%)	14/100 (14%)	2/100 (2%)	73/100 (73%)

Anaphylaxis, local sensitivity and other data is presented as the number of patients with positive skin-prick tests (SPTs) and specific symptoms compared to the total number of positive SPTs for that specific food. *Local sensitivity includes perioral pruritus or hand pruritus.

patients. While the reason for this is not known, the majority of the latex-allergic patients were female health-care workers.

It was of interest that sensitization to latex was associated with local clinical reactions to foods, especially perioral sensitivity and hand pruritus when handling specific foods. A similar crossreactivity has been documented between food allergens and birch and ragweed pollens [17,18]. Local allergy to raw potato has previously been reported in patients who do not appear to react clinically to cooked potatoes [19]. This agrees with the results obtained when investigating allergy to other fruits and vegetables where heat appears to denature the allergenic proteins.

It should also be noted that the majority of positive food skin tests (73/100) were not associated, clinically, with symptomatic allergy. Continued follow-up of these patients should clarify the significance of their positive tests but presently these would represent asymptomatic sensitivities. However, eight patients had 11 systemic anaphylactic episodes to banana, avocado, tomato or kiwi confirming the significance of their positive skin tests. The foods that we observed to most frequently cause anaphylaxis (avocado and banana) were the same as those observed by Blanco *et al.* [12].

The skin-test data indicate that simultaneous sensitization to latex and food allergens is common. This unique sensitization appears to develop among latex allergic patients who may then develop clinical allergy to food, and perhaps, among food allergy patients who then may react to latex [7,10]. Besides the patients and controls described here, we have tested six food-allergic patients with IgE mediated urticaria and anaphylaxis but with no regular latex exposure. These patients were skin-test positive to a fraction of HPLC-separated non-ammoniated latex proteins corresponding to a molecular weight of 30–46 kDa (manuscript in preparation). Crossreacting epitopes that are shared by proteins in these foods appear to be present on the proteins in this HPLC fraction of latex.

It has been postulated that there are common epitopes in these diverse groups of plants that are immunologically crossreactive and results in crossreacting food allergy. Previously, we have identified the hevein preprotein as an allergen in latex [5]. The hevein preprotein is synthesized as a 20 kDa polypeptide that undergoes post-translational processing and is cleaved into the 5 kDa hevein and a 14 kDa C-domain protein. Hevein is a chitin binding protein that is known to be important for latex agglutination [20]. Hevein has structural homology with ragweed allergens and wheat germ agglutinin. The 14 kDa C domain of hevein preprotein has homology with the potato stress proteins WIN 1 and WIN 2 and thus potentially represents the crossreactive allergen [21]. However, it is unlikely that the hevein

preprotein is the only allergen in latex with structural homology to other plant proteins.

Previously, we have shown that latex allergic patients recognized protein bands at 46 and 110 kDa that had the same N-terminal sequence [5]. In this report, we have further characterized the 46 kDa latex protein as a new latex protein allergen having sequence homology with another plant protein. The 46 kDa protein reacts with IgE from latex allergic patients and was found to have both N-terminal (60%) and internal sequence (59%) homology with patatin. Patatins are storage proteins found in plant families such as potato and tomato [15,16]. The 46 kDa allergen in latex appears to represent a *Hevea brasiliensis* homologue of patatin. The structural homology of the 46 kDa allergen to patatin may help to explain the unexpected finding of clinical reactivity to potato and tomato in latex allergic patients. Allergy to potato has been reported [17,20], but patatin has not been identified as an allergen.

Immunological inhibition assays have been used to demonstrate crossreactivity of latex with banana, chestnut, and avocado [10,11,23,24]. Using immunoblot inhibition experiments, we were able to demonstrate anti-genic cross-reactivity with several potato proteins. We were unable to completely block the reactivity to the 46 kDa protein identified as a patatin homologue, suggesting only limited crossreactivity with this protein. This is not unexpected since the latex 46 kDa protein demonstrated only 60% homology with patatin and would likely contain epitopes not present in the potato protein. Studies to identify the antigenic and allergenic epitopes in the 46 kDa protein are in progress. While patatin may be one crossreacting protein, it was not the only crossreacting antigen as several, yet unidentified proteins were also inhibited. The cross-reactivity of multiple proteins helps to explain the surprisingly high level of food reactivity in latex-allergic patients.

Our findings of extensive immunological crossreactivity between latex and foods are clinically important. Clearly, there is the potential for serious allergic reactions to foods in patients sensitized to latex. There is also the possibility of allergic reactions to latex in patients with food allergy. Presently, we recommend a careful history of food allergy in all latex allergic patients. Skin tests with extracts of potentially crossreacting foods would identify a risk for allergic reactions. Further studies are needed to evaluate the prevalence and significance of positive latex skin tests in patients with food allergy.

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