

Association between sensitization to natural rubber latex and papain

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Several studies have documented clinical and immunologic cross-reactivity between natural rubber latex (NRL) and a variety of fruits, mainly banana, avocado, and kiwi.^{1,2} We fortuitously observed that a nurse with NRL allergy also showed skin reactivity to papain, although she had had no known exposure to this enzyme, which is extracted from the latex of the papaya tree (*Carica papaya*).

Papain is widely used in the food industry (meat tenderizer, beer clearing agent), as well as in pharmaceuticals (local treatment of necrotic ulcers, digestive aid, cleaning agent for contact lenses, chemonucleolysis) and cosmetic products.³⁻⁵ The latex of *C. papaya* and commercial preparations of papain contain a number of characterized enzymes, including cysteine proteinases (papain itself, chymopapain, caricain, and endoproteinase Gly-C; molecular weight, 23 to 24 kd) and chitinase (molecular weight, 28 kd).⁶ Papain has been documented as causing IgE-mediated allergic reactions after domestic and occupational exposure.³⁻⁵ Quarre et al.⁷ recently reported an association between allergy to NRL and papain in a subject with occupational exposure to both allergens.

The purpose of this study was to assess the prevalence of sensitization to papain among health care workers with and without NRL allergy.

METHODS

Subjects

Thirty of 31 consecutive health care workers, who were diagnosed as having latex allergy in our center over

Abbreviations used

CI:	Confidence interval
NRL:	Natural rubber latex
SIC:	Specific inhalation challenge
SPT:	Skin prick test

a 2-year period, participated in this study. This group included 22 nurses, two physicians, three laboratory technologists, two members of hospital cleaning staff, and one medical secretary. The control population included 148 randomly selected health care workers (133 nurses and 15 physicians) without demonstrable allergy to NRL. The participants completed a standardized medical and occupational questionnaire,⁸ which also contained specific questions regarding exposure to papain-containing products.⁵ They underwent skin prick tests (SPTs) with common allergens, NRL, and papain extracts. Subjects with NRL allergy were given specific inhalation challenge (SIC) with NRL gloves, and 16 of them also agreed to undergo SIC with papain. The study protocol was approved by the local ethics committee.

SPTs

All participants were skin-tested with diluent, histamine chlorhydrate (1 mg/ml), and the following extracts: (1) seven common inhalant allergens including *Dermaphagoides pteronyssinus*; cat and dog danders; mixtures of tree, grass, and weed pollens; and a mixture of molds (Stallergènes SA, Brussels, Belgium); (2) a commercial extract of NRL (Stallergènes SA); (3) an extract of NRL gloves⁸; (4) crude papain (P 3375; Sigma Chemicals, Bornem, Belgium) diluted 1:100 in phosphate-buffered saline; and (5) the whole protein fraction of papaya (protein concentration, 5 mg/ml), obtained by ion-exchange chromatography.⁶

NRL-sensitive subjects underwent additional SPTs with each papaya enzyme (1 mg/ml) purified to homogeneity by using a combination of ion-exchange and hydrophobic chromatographic techniques. These purified enzymes included papain itself (EC 3.4.22.2), chymopapain (EC 3.4.22.6), caricain (EC 3.4.22.30 or papaya proteinase III or W), endoproteinase Gly-C (EC 3.4.22.25 or papaya proteinase IV), and chitinase (formerly called *papaya lysozyme*).

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Skin reactions were read after 15 minutes and were considered positive when the mean wheal diameter was 3 mm or greater as compared with the negative control. Atopy was defined by a positive SPT response to at least one common allergen.

Inhalation challenges

SICs with NRL gloves were carried out in a 5 m³ challenge room according to a previously described protocol.⁸ On a control day, the subjects were asked to handle vinyl gloves for 1 hour, and spirometry was assessed for at least 6 hours after the end of exposure to confirm that spontaneous fluctuations in FEV₁ were less than 10%. On the next day, the subjects were challenged with NRL gloves used at work (Triflex powdered gloves; Baxter Healthcare Corporation, Valencia, Calif.). The content of extractable protein of these gloves was 51 mg/gm.⁹ The subjects wore vinyl gloves to prevent any direct cutaneous contact with NRL. The duration of exposure was gradually increased until FEV₁ fell by more than 20% or until a cumulative exposure of 4 hours was completed. Results of SICs were considered positive when the subjects showed a fall of 20% or greater in FEV₁.

SICs with papain were performed by pouring crude papain powder diluted 1:100 in lactose powder from one tray to another for a maximum of 120 minutes. A control exposure to lactose powder, performed in three subjects with positive SIC responses to papain, did not elicit bronchial response.

RESULTS

Health care workers with a history of NRL allergy and positive SPT responses to NRL (28 women, 18 atopic subjects) had a mean age of 32 ± 4 years and a mean duration of work exposure of 9 ± 4 years. Symptoms related to NRL exposure included contact urticaria ($n = 29$), rhinitis ($n = 29$), and anaphylactic reactions ($n = 3$). Ten subjects reported clinical allergy to fruits including banana ($n = 6$), kiwi ($n = 5$), and avocado ($n = 3$). Twenty-two subjects had NRL-induced asthma, as demonstrated by SICs (Table I). The control population of health care workers without allergy (112 women, 39 atopic subjects) had a mean age of 33 ± 7 years and a mean duration of work exposure of 10 ± 7 years.

Crude papain extract elicited a positive SPT response in 18 of 30 health care workers with NRL allergy (60%; 95% confidence interval [CI], 41% to 77%) (Table I) and in one of 148 control workers without NRL allergy (0.7%; 95% CI, 0% to 4%; Fisher's exact test, $p < 0.001$).

Among the 30 subjects with NRL allergy, sensitization to papain was strongly associated with a history of allergy to fruits. Nine of 10

subjects who reported fruit allergy showed positive SPT responses to papain as compared with none of the 20 subjects without fruit allergy ($p < 0.001$). Sensitization to papain was also more frequent in subjects who showed positive responses to SIC with NRL gloves (16 of 22) than in those who did not (2 of 8, $p = 0.05$). Sensitization to papain was related neither to atopy nor to the questionnaire-derived score of exposure to papain. There was no association between skin reactivity to individual inhalant allergens and either NRL or papain.

There was agreement between the results of SPT with crude papain and the extract containing all papaya proteins, indicating that positive skin reactions were not related to some nonprotein constituent. Sixteen of 18 subjects sensitized to crude papain and the protein fraction of papaya demonstrated a positive SPT response to at least one of the purified papaya enzymes (Table I) including papain ($n = 16$), chymopapain ($n = 1$), endoprotease Gly-C ($n = 4$), and chitinase ($n = 3$).

SICs with crude papain elicited an asthmatic reaction in nine of 11 subjects with positive SPT responses to papain and in none of five subjects with negative SPT responses (Table I). The subjects who had an asthmatic reaction to crude papain had baseline histamine PC₂₀ values (geometric mean, 0.54 mg/ml) similar to those who did not (geometric mean, 0.28 mg/ml).

DISCUSSION

This study showed a strong association between NRL allergy and sensitization to papaya enzymes, of which papain itself appeared to be the principal sensitizing protein. The prevalence of positive SPT responses to papain in our control group without NRL allergy (0.7%; 95% CI, 0% to 4%) was similar to the findings of previous investigators looking at the general population (3%; 95% CI, 2% to 4%).³⁻⁵ Among health care workers with NRL allergy, skin reactivity to papain correlated with allergy to fruits and, to a lesser extent, with the presence of NRL-induced asthma.

The clinical relevance of these findings remains uncertain. Immunologic cross-reactivity between NRL and botanically unrelated fruits has been documented even in subjects without a history of allergic reactions to these fruits.² Two of our subjects sensitized to papain reported having ingested papaya fruit in the preceding year without experiencing allergic symptoms. An allergic reaction possibly related to papain was documented in

TABLE I. Results of SPTs and inhalation challenges in subjects with NRL allergy

SPTs (mean wheal diameter, mm)														
Subject No.	Atopy	NRL	NRL gloves	Crude papain	Whole protein fraction of papaya	Purified papaya enzymes					Baseline FEV ₁ (% pred)	Baseline PC ₂₀ * (mg/ml)	Inhalation challenge†	
						Papain	CP	CC	EP	CT			NRL gloves	Crude papain
Subjects with NRL-induced asthma														
1	+	5	7	6	7	5	—	—	—	4	120	16.00	E (140)	E (10)
2	+	3	8	4	6	4	—	—	—	—	101	0.70	E (120)	ND
3	+	4	11	10	8	6	—	—	—	—	85	<0.03	D (20)	E (3)
4	—	3	6	10	7	6	—	—	—	—	93	0.35	D (90)	E (20)
5	—	4	7	5	6	5	4	—	3	—	91	<0.03	D (70)	D (5)
6	—	5	6	6	8	3	—	—	—	—	95	1.77	E (180)	E (20)
7	+	4	6	4	6	4	—	—	3	—	104	4.00	E (120)	E (15)
8	—	4	7	7	6	5	—	—	4	—	96	0.28	E (120)	E (10)
9	+	6	6	7	8	6	—	—	—	—	96	1.00	E (30)	E (5)
10	—	7	7	6	5	4	—	—	—	3	93	0.08	D (70)	ND
11	—	9	ND	4	5	4	—	—	—	—	93	0.43	E (110)	ND
12	+	6	ND	3	6	5	—	—	—	—	100	0.07	D (20)	ND
13	+	7	ND	5	6	4	—	—	4	4	78	0.26	E (120)	ND
14	+	3	8	5	6	4	—	—	—	—	85	0.05	D (20)	ND
15	+	5	5	4	6	5	—	—	—	—	90	0.36	E (120)	ND
16	+	4	6	4	5	3	—	—	—	—	119	0.40	E (120)	E (60)
17	+	4	8	—	—	—	—	—	—	—	100	1.38	E (120)	ND
18	—	3	9	—	—	—	—	—	—	—	116	2.90	E (180)	—
19	+	3	5	—	—	—	—	—	—	—	101	0.08	E (210)	—
20	+	6	8	—	—	—	—	—	—	—	84	0.24	E (210)	—
21	—	4	10	—	—	—	—	—	—	—	87	0.87	E (100)	ND
22	—	4	6	—	—	—	—	—	—	—	99	1.00	L (180)	—
Subjects without NRL-induced asthma														
23	+	4	5	4	3	—	—	—	—	—	87	<0.03	—	—
24	+	6	8	3	3	—	—	—	—	—	94	0.16	—	—
25	+	4	6	—	—	—	—	—	—	—	108	0.50	—	—
26	—	4	4	—	—	—	—	—	—	—	96	8.25	—	ND
27	+	5	7	—	—	—	—	—	—	—	116	2.00	—	ND
28	+	4	8	—	—	—	—	—	—	—	104	2.44	—	ND
29	—	3	4	—	—	—	—	—	—	—	103	7.50	—	ND
30	—	4	5	—	—	—	—	—	—	—	119	9.10	—	ND

CP, Chymopapain; CC, caricain; EP, endoproteinase Gly-C; CT, chitinase; E, early asthmatic reaction; D, dual asthmatic reaction; L, late asthmatic reaction; ND, not done.

*Histamine PC₂₀ was assessed by inhalation of doubling concentrations of histamine (from 0.03 to 16 mg/ml) at tidal breathing for 2-minute periods and considered to reflect significant nonspecific bronchial hyperresponsiveness when less than 16 mg/ml.

†Pattern of asthmatic reaction and duration of challenge exposure between brackets.

only one subject (no. 3), who experienced urticaria caused by a cosmetic cream. Results of SICs, however, indicated that papain can induce asthmatic reactions in most subjects with skin reactivity. Conversely, the occurrence of sensitization and allergic reactions to NRL in workers exposed to papain should be evaluated.

Awareness of an association between allergy to NRL and papain may be important in view of their widespread use in both domestic and occupational settings, particularly in light of the increasing inci-

dence of NRL allergy. The possibility of cross-reacting allergenic proteins warrants further investigation with in vitro methods. We did not identify relevant (>5 amino acids) sequence homology between papain and described latex proteins, suggesting that conformational epitopes could be involved in immunologic cross-reactivity.

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Safety and efficiency of an accelerated method for venom skin testing

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In the current delivery of quality medical care, time spent on diagnostic procedures involving patients represents a significant proportion of total cost. Patients who are allergic to Hymenoptera stings require efficient, safe methods of evaluation.

Hymenoptera venom skin testing has historically been a lengthy process. The manufacturer's package insert recommends that an initial prick test be performed at a concentration of 1.0 µg/ml, and if results are negative, serial intradermal skin tests are performed. Sequential concentrations recommended are 0.001 µg/ml, 0.01 µg/ml, 0.1 µg/ml, and 1 µg/ml. This approach to Hymenoptera skin testing is based on results of early studies.¹⁻³ The application of sequential skin tests, their measurement, and recording of results can take 2 hours to complete.

For the past 15 years, we have used an accelerated procedure for Hymenoptera venom testing. The ini-

tial skin prick test concentration is 1.0 µg/ml; intradermal tests are then performed with venom concentrations of 0.2 µg/ml and 1 µg/ml, placed simultaneously on the patient's skin. We report the results of this accelerated Hymenoptera venom testing over a 10-year period.

METHODS

Lyophilized venoms of honeybee, wasp, yellow jacket, yellow hornet, and white-faced hornet were purchased from ALK Laboratories, Inc. (Milford, Conn.) or Miles, Inc. (Pittsburgh, Pa.). Diluent control was Sorenson's solution containing 0.5% normal serum albumin and 0.4% phenol. The positive intradermal control was a histamine concentration of 0.05 mg/ml. Prick testing was done with a disposable bifurcated needle. Intradermal testing was performed with a 0.5 ml syringe and a 27-gauge needle (5/8 inch).

Venoms reconstituted with Sorenson's solution were diluted to 10 µg/ml, 1 µg/ml, and 0.2 µg/ml concentrations. Prick testing was done with venom at a concentration of 1 µg/ml, as well as with a diluent control. Test sites were measured and recorded after 15 minutes. If the response was negative, intradermal testing was performed. Venoms at concentrations of 0.2 µg/ml and 1 µg/ml were injected simultaneously in separate sites, a volume of 0.03 ml, creating a 5 × 5 mm wheal. Histamine and diluent controls were injected to create 2 × 2

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