

Allergenic Components of Indian Jujube (*Zizyphus mauritiana*) Show IgE Cross-Reactivity with Latex Allergen

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Key Words

Indian jujube · Latex-fruit syndrome · Allergy · Cross-reactivity · IgE

Abstract

Background: 'Latex-fruit syndrome' has been well documented. A prevalence of latex allergy among medical workers of 6.8–8.6% had been reported in Taiwan. However, there has been no study to determine the importance and type of fruit hypersensitivity in latex-allergic patients in Taiwan. This study aimed to identify the allergenic components of Indian jujube (*Zizyphus mauritiana*) and characterize the cross-reactivity of specific IgE antibodies to latex allergen. **Methods:** Crude extracts were prepared from Indian jujube and from ammoniated natural rubber latex, and six medical workers and one patient with a history of fruit allergy underwent skin testing with routine allergens, latex, Indian jujube and other fruits. Sera from two Indian jujube skin test-positive latex-allergic subjects were used for allergen-specific IgE, immunoblotting, immunoblot inhibition and enzyme-linked immunosorbent assay (ELISA) inhibition studies. **Results:** Both patients had positive skin test responses and specific IgE assays to Indian jujube and latex extracts. Immunoblotting revealed that IgE from both subjects bound to

a 42-kD latex protein and a 42-kD Indian jujube protein. In addition, IgE from one subject bound to a 30-kD Indian jujube protein. Preincubation of atopic sera with Indian jujube or latex extract demonstrated absent and/or marked inhibition of IgE binding. Moreover, anti-Indian jujube protein antibody-based ELISA was able to detect latex extracts. **Conclusions:** Our results add to findings regarding the 'latex-fruit syndrome' described in the literature, and further study of the cross-reacting allergens identified in Indian jujube may help to elucidate the mechanisms underlying this syndrome.

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Introduction

It is now well established that latex allergy is often combined with allergy to certain plant foods, especially fruits. In 1994, the existence of a 'latex-fruit syndrome' was proposed based on the clinical observation of an unexpectedly high rate of fruit allergy in patients allergic to latex [1, 2]. Since then, several reports have documented the association between latex allergy and sensitization to banana, avocado, chestnut, passion fruit, fig, pineapple, kiwi, potato, papaya, peach, grape, orange, tomato, buckwheat, melon, celery and peanut [3]. Al-

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though there is no taxonomic relationship among the vegetal species implicated in the latex-fruit syndrome, its existence has been fully confirmed by different authors [3–5].

Despite its importance in the elicitation of hypersensitivity reactions, latex allergy has not been closely studied in Taiwan. In 1997, Lai et al. [6] reported a prevalence of latex allergy of 6.8% (70/1,021) among personnel of the National Taiwan University Hospital. There has been no study to determine the importance, type and frequency of fruit hypersensitivity in latex-allergic patients in Taiwan. Recently, Chen and Lan [7] reported that 8.6% (26/302) of medical workers in Taichung had immediate allergic reactions to latex, and 26.2% (7/26) of the latex-allergic subjects had latex-fruit syndrome. Among those subjects, 3 (dentist and laboratory technicians) were allergic to pineapple, 1 (physician) was allergic to wax apple, 1 (laboratory technician) was allergic to kiwi and peach and 2 (laboratory technician and student) were allergic to Indian jujube. Indian jujube, a native species of the Yunnan province in southern China, is a sweet fruit which grows in a warm climate, and is abundantly cultivated in southern Taiwan. It is commonly becoming an increasingly popular fruit during its season in late autumn. Our present study identifies the allergenic components of the Indian jujube which are also cross-reactive with latex allergen.

Subjects and Methods

Extraction of Natural Rubber Latex and Indian Jujube

Ammoniated natural rubber latex was centrifuged at 10,000 *g* for 30 min, and the centrifugations were repeated 4 more times until no more rubber coagulated materials were found. The supernatant was then filtered through a 0.45- μ m filter and dialyzed with phosphate-buffered saline (PBS) overnight at 4°C, concentrated using Amicon Ultra PL-10 (Millipore Corporation, Bedford, Mass., USA) and stored at –70°C. Indian jujube extract was prepared by a modification of the low-temperature procedure [8]. The fresh fruit (100 g) was mechanically homogenized in 200 ml of precooled acetone, filtered, washed twice with acetone-ether (1:1, vol/vol) and lyophilized. The lyophilized materials (10 g) were stirred for 1 h at 4°C with 200 ml of PBS (10 mM, pH 7.4) containing 2 mM ethylenediamine tetraacetic acid, 5 mM diethyldithiocarbamic acid, 0.5 mM benzamidine hydrochloride and 0.2 mM phenylmethyl sulfonyl fluoride. The mixture was centrifuged at 10,000 *g* at 4°C for 30 min, and the supernatant was concentrated using Amicon Ultra PL-10 (Millipore) and stored at –70°C. The protein concentration was determined by the Coomassie brilliant G-250 protein-dye binding method of Bradford [9].

Subjects

A 26-year-old male student (patient 1) with atopic dermatitis and asthma since childhood and a healthy 38-year-old female laboratory

technician (patient 2) who presented with recurrent episodes of angioedema and throat tightness after ingestion of Indian jujube were studied. Patient 1's dermatitis had worsened in adulthood to the point that it limited his daily activities and overall performance status. Though he could not recall any immediate allergic reactions to food, a survey of possible food allergies was performed because of his severe atopic dermatitis. Patient 2 was nonatopic before working as a laboratory technician. She had worked as a laboratory technician since the age of 23 years. After wearing latex gloves daily for 8 years, clinical symptoms gradually appeared. Around 2 years after the development of her sensitivity to latex, immediate hypersensitivity occurred with ingestion of Indian jujube. Patient 2 noticed pruritic dermatitis over her hands after wearing latex gloves at work. She also reported eye irritation and rhinorrhea at work, which resolved after work hours and on the weekends. Within 20 min of eating Indian jujube, she experienced periorbital edema, lip and tongue edema and throat tightness.

Skin Testing

The patients underwent skin tests with routine allergens [house dust, *Aspergillus* species, *Penicillium* species, *Cladosporium* species, *Candida albicans*, grass, weed, cat and dog dander (ALK, Hørsholm, Denmark)]. Skin tests were also performed for latex and foods, i.e. apple, carrot, tomato, orange, peach, kiwi, banana, mango, apricot, melon, passion fruit, wheat, peanut, soybean, celery, mustard, onion and walnut extracts (Stallergene, France). Extracts of Indian jujube and latex prepared in the laboratory (100 μ g/ml in PBS containing 50% glycerol) were also used for skin tests. Allergens were applied to the testheads with a Quick dispenser (Greer Laboratories, Lenoir, N.C., USA), and an epicutaneous skin test was performed. PBS containing 50% (vol/vol) glycerol and 1.0 mg/ml histamine were included as negative and positive controls, respectively. Reactions were read at 15 min and graded against controls. Erythema was measured. The mean diameters of the wheal and erythema were graded against the controls. A reaction comparable to the negative control was considered negative. Responses of a skin wheal 2.0 mm or erythema 5.0 mm larger than the negative control were graded 1+, and responses of a skin wheal 4.0 mm larger or erythema 10 mm larger than the negative control were graded 2+. A reaction comparable to the positive control, which typically resulted in a wheal of 7–10 mm and erythema greater than 20.0 mm, was graded 3+. A reaction greater than the positive control, which was sometimes associated with pseudopodia and itching, was graded 4+.

Allergen-Specific IgE

Diagnostic measurements of total and allergen-specific IgE were performed with the CAP-FEIA system (Pharmacia Diagnostic, Sweden). Specific IgE to laboratory-prepared latex and Indian jujube extracts was measured by using a fluoroallergosorbent test (FAST) according to the instructions of the manufacturer (IgE FAST-Plus Test, 3M Diagnostic System Inc.) as previously described [10] and enzyme-linked immunosorbent assay (ELISA). ELISA was performed as described below without preincubation of serum with inhibitor.

SDS-PAGE and Immunoblotting

Crude latex or Indian jujube extract was suspended in SDS sample buffer containing 2-mercaptoethanol for SDS-PAGE. Thirty microliters of a 70 μ g/ml solution of sample proteins were loaded on a 5% polyacrylamide stacking gel above a 12% separating gel, and the

Table 1. Personal history, results of positive skin tests and total and specific IgE determination in our two patients

Parameter	Patient No. 1 (26-year-old male)	Patient No. 2 (38-year-old female)
Indian jujube allergy symptoms	atopic dermatitis	angioedema
Latex allergy symptoms	dermatitis	rhinoconjunctivitis
Concomitant allergic diseases	atopic dermatitis, asthma	none
Positive skin test results	hd (4+), as (2+), ps (3+), gr (1+), we (3+), latex (3+), wheat (2+), mango (3+), orange (2+), banana (2+), latex ¹ (4+), Indian jujube ¹ (2+)	latex (2+), latex ¹ (3+), Indian jujube ¹ (4+)
Total IgE, kU/l	12,500	352
FAST ² , kU/l	latex: 3.8 (3); Indian jujube ¹ : 14 (4)	latex: 1.2 (2); Indian jujube ¹ : 0.65 (1)
CAP ² , kU/l	latex: 25.5 (4); wheat: 30 (4); mango: 71.2 (5); orange: 45.1 (4); melon: 33.1 (4); apple: 63.1 (5)	latex: 1.48 (2); wheat: ND; mango: ND; orange: ND; melon: ND; apple: ND

hd = House dust; as = *Aspergillus* species; ps = *Penicillium* species; gr = grass pollen; we = weed pollen; ND = not determined.

¹ Laboratory prepared extracts.

² FAST and CAP classes are shown in parentheses.

gel was run with discontinuous buffer according to the method of Laemmli [11], followed by standard immunoblotting [12]. Immunologic detection was performed using atopic human sera as described previously [13]. Blots were probed with the atopic human serum (1:30). Immunodetection was performed using a horseradish peroxidase chemiluminescence kit according to the instructions of the manufacturer (Pierce, Rockford, Ill., USA).

Inhibition Studies

In immunoblot inhibition, serum was mixed with an equal volume of either latex or Indian jujube extract (150 µg/ml) at 4 °C overnight, and immunoblotting was then performed as described above. In ELISA inhibition, optimal concentrations of antigen and conjugate were determined by checkerboard titration. Latex or Indian jujube extract was coated onto wells in triplicate and incubated at 37 °C for 2 h. After incubation, wells were then washed with PBS containing 0.05% Tween 20, and 1.0% normal goat serum was added and incubated for 2 h at room temperature. Wells were washed and incubated overnight at 4 °C with a 1:10 dilution of serum which had been preincubated for 2 h at room temperature with different concentrations (10–160 µg/ml) of latex or Indian jujube extract. After washing, antibody binding was detected using peroxidase-labeled goat anti-human IgE along with 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) containing 0.03% H₂O₂ as a substrate. The optical density was determined at 415 nm on an SLT SPECTRA Reader (SLT Labinstruments, Salzburg, Austria). Latex or Indian jujube extract was used as inhibitor for the positive control, and preincubation of inhibitor with latex and Indian jujube skin test-negative allergic sera was used as the negative control. Results are expressed as the percentage inhibition of the reaction of antibody without any inhibitor.

Anti-Indian Jujube Antibody

Antibodies against Indian jujube extract were raised in rabbits. Young adult New Zealand white rabbits were immunized with multiple intradermal injections of 2.0 mg/ml crude extract of Indian jujube with an equal volume of complete Freund's adjuvant (Difco

Laboratory, Detroit, Mich., USA). The primary immunization was followed by monthly intradermal injection of 0.5 mg/ml booster mixed with Freund's incomplete adjuvant. Antisera were pooled and precipitated with 18% sodium sulfate followed by a 14% sodium sulfate precipitation. The partially purified anti-fruit IgG was dialyzed against Tris-HCl buffer, pH 8.0, at 4 °C and stored.

Detection of Proteins in Latex Extracts by ELISA with Anti-Indian Jujube Antibody

The reactivities of latex extracts were evaluated in a direct binding ELISA. ELISA was performed essentially as described previously [13]. Optimal concentrations of antigen and conjugate were determined by checkerboard titration. Microtiter plates were coated either with 100 µl of Indian jujube extract (50 µg/ml), a commercial skin testing latex (Stallergene), ammoniated latex extracts (50 µg/ml) or unimmunized rabbit IgG (50 µg/ml) in triplicate and incubated for 2 h at 37 °C. After washing, anti-Indian jujube antibody (5.0 mg/ml, 1:3,000) was added and incubated for 2 h at room temperature. Antibody binding and the optical density were determined as described above.

Results

Medical history, skin test results and IgE determinations of the two patients are summarized in table 1. In both cases in our study, positive skin tests to Indian jujube were demonstrated. Either CAP or FAST studies detected increased specific IgE levels to Indian jujube and latex in both patients. In case 1, results revealed that the different types of fruit-specific IgE are all class 4 or 5. Case 2 has typical 'latex-fruit syndrome'. These results are in concordance with the patients' clinical symptoms. Moreover, both atopic sera showed positive human IgE binding, and

Fig. 1. Binding of IgE antibodies to Indian jujube (**A**) and latex (**B**) as measured by ELISA. Two atopic sera (patients 1 and 2) and serum samples from Indian jujube and latex skin test-negative atopic (patient 3) and healthy nonallergic subjects (patient 4) were analyzed. Microtiter plates were coated with 30 µg/ml Indian jujube or latex extract, and results are the average of experiments performed in triplicate. OD = Optical density.

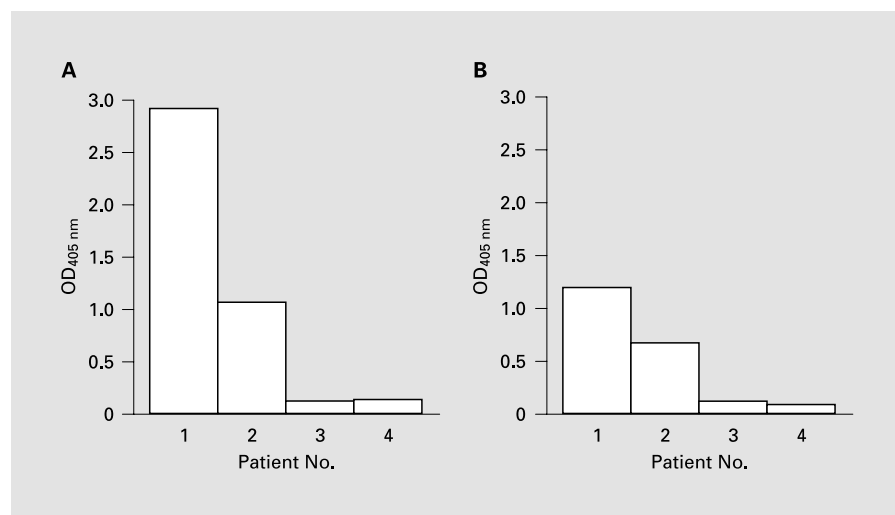
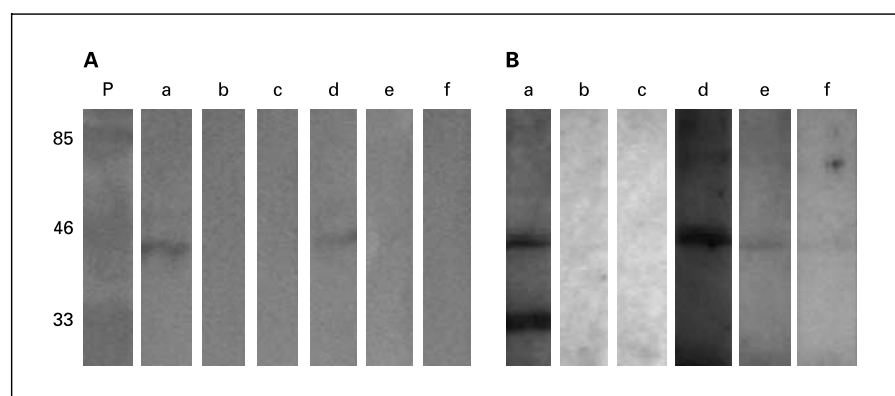


Fig. 2. Cross-reactivity by immunoblot inhibition. Binding of IgE from patient 1 (lanes a, b and c) and patient 2 (lanes d, e and f) to latex (**A**) and Indian jujube (**B**) allergens, inhibited by 150 µg/ml proteins from latex (lanes b and e) or Indian jujube (lanes c and f). Lanes a and d: no inhibitor; lane P: protein markers in kD.



no reactivity was detected for Indian jujube and latex skin test-negative atopic and healthy nonallergic subjects using ELISA (fig. 1).

Indian Jujube and Latex Proteins and Allergens

SDS-PAGE profiles of Indian jujube and latex extracts revealed predominant components with apparent molecular weights of 42, 35, 30, 28 and 14 kD, and 42, 35, 31, 28 and 14 kD, respectively (data not shown). Immunoblotting results demonstrated human IgE binding to both a 42-kD latex protein (fig. 2A, lanes a and d) as well as a 42-kD Indian jujube protein (fig. 2B, lanes a and d). Additionally, IgE from one of the atopic subjects (patient 1) bound to a 30-kD Indian jujube protein (fig. 2B, lane a).

Immunoblot Inhibition Studies

In homologous immunoblot inhibition, IgE binding of 42-kD proteins was suppressed when sera from both patients were preincubated with latex proteins (fig. 2A,

lanes b and e). Preincubation of serum from patient 1 with Indian jujube proteins inhibited the binding of 30- and 42-kD proteins of Indian jujube (fig. 2B, lane c). However, in patient 2, IgE binding of the 42-kD protein was markedly weakened when serum was preincubated with Indian jujube proteins (fig. 2B, lane f). In heterologous immunoblot inhibition, preincubation of serum from patient 1 with either Indian jujube or latex proteins inhibited IgE binding of 30- and 42-kD proteins (fig. 2A, lane c, fig. 2B, lane b). On the other hand, preincubation of serum from patient 2 with Indian jujube protein inhibited the IgE binding of the 42-kD latex protein (fig. 2A, lane f). The IgE binding of the 42-kD Indian jujube protein was weakened when sera were preincubated with latex proteins (fig. 2B, lane e).

ELISA Inhibition Studies

To confirm the immunoblot inhibition, ELISA inhibition studies were performed. Preincubation of Indian

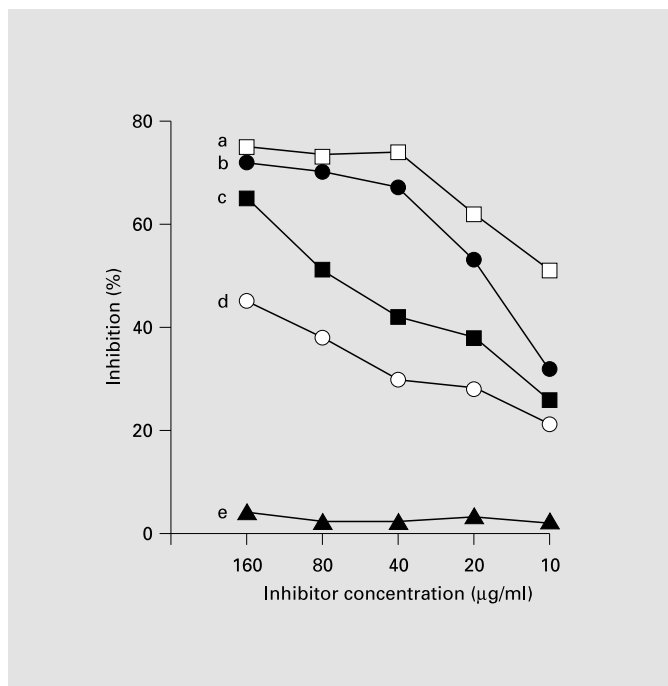


Fig. 3. Inhibition of binding of IgE from patients 1 (a and d) and 2 (b and c) to Indian jujube by ELISA. Inhibitors: a and b: Indian jujube; c and d: latex; e: latex and Indian jujube skin test-negative serum. Microtiter plates were coated with 30 µg/ml Indian jujube extract, and results are the average of experiments performed in triplicate.

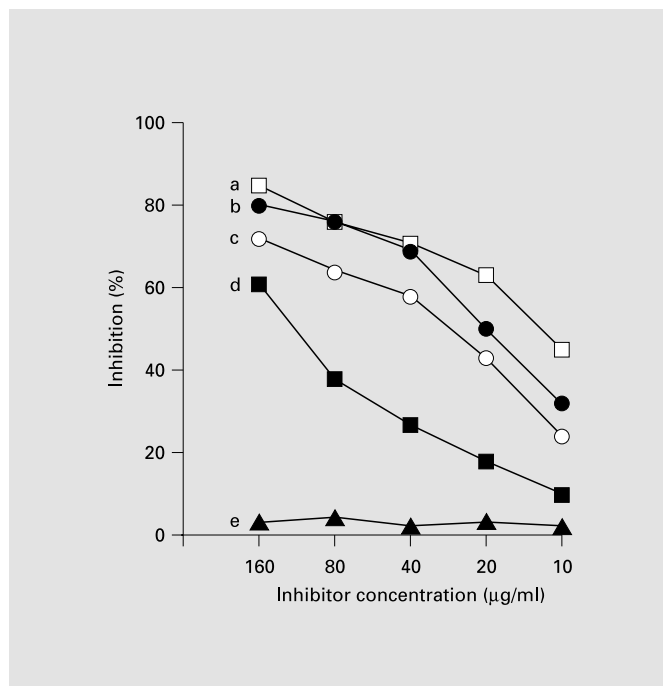


Fig. 4. Inhibition of binding of IgE from patients 1 (a and d) and 2 (b and c) to latex by ELISA. Inhibitors: a and b: Indian jujube; c and d: latex; e: latex and Indian jujube skin test-negative serum. Microtiter plates were coated with 30 µg/ml latex extract, and results are the average of experiments performed in triplicate.

jujube or latex with both atopic sera resulted in marked inhibition of the IgE binding to Indian jujube (fig. 3). In addition, preincubation of Indian jujube or latex with both atopic sera resulted in marked inhibition of the IgE binding to latex (fig. 4). No significant inhibition was observed when buffer was used as inhibitor.

Detection of Latex Proteins by Anti-Indian Jujube Antibodies

A polyclonal antibody-based ELISA using anti-Indian jujube was performed for the latex extract and skin test reagent proteins. The response of Indian jujube to anti-Indian jujube was dose dependent, and no Indian jujube proteins were detected when preimmunized rabbit serum was substituted for anti-Indian jujube (data not shown). The optical densities at 415 nm for Indian jujube, commercial skin testing latex, ammoniated latex extract and unimmunized rabbit IgG were 1.462, 1.244, 0.432 and 0.018, respectively (the average of experiments performed in triplicate).

Discussion

Several latex allergens have been cloned and sequenced [14]. Hev b 6 containing prohevein/hevein domains is a major natural rubber latex allergen which shares high sequence homology with pathogenesis-related proteins (PR-3 and PR-4) [15, 16]. Recently, class I chitinases (25–35 kD) with an N-terminal hevein-like domain have been identified in avocado, chestnut and banana as the main allergens cross-reacting with latex [17–19]. Moreover, a survey of 20 different food extracts indicated that putative class I chitinases also seem to be relevant cross-reacting proteins linked to latex-fruit allergy in cherimoya, passion fruit, kiwi, papaya, mango, tomato and wheat [20]. Major putative allergens of 30–35 kD are most frequently detected in avocado, chestnut, banana and other fruits when using sera from patients allergic to latex [21–23]. These data point to a central role of the class I chitinases, particularly their N-terminal hevein-like domains, as the main allergens responsible for the cosensitizations between latex and fruits.

Recently, a brief report on one case of anaphylactic reactions to azufaifa fruit (*Ziziphus jujuba*) was published [24]. The binding of IgE to a 30-kD allergen of azufaifa fruit can be inhibited by latex extract and weakly inhibited by recombinant chestnut class I chitinase extract. The 30-kD allergens identified in Indian jujube from our patient 1 and from azufaifa fruit have a molecular weight similar to avocado, chestnut and banana class I chitinases. Our results and others lead to the supposition that the catalytic domains of class I chitinases could be involved in cross-reactions. We cannot determine the N-terminal sequences of the 30- and 42-kD components of Indian jujube due to the blocking of the N-terminus. As the Indian jujube proteins and the 30-kD protein of azufaifa fruit have not been sequenced, the sensitization could be due to another allergen, as IgE from one of our patients

(patient 2) recognized only the 42-kD protein of Indian jujube.

In summary, two 30- and 42-kD allergens from Indian jujube and a 42-kD allergen from latex were identified using patients' IgE by immunoblotting, and the homologous and heterologous inhibition studies demonstrated marked inhibition as defined by immunoblot and ELISA inhibitions. Moreover, anti-Indian jujube protein antibody-based ELISA was able to detect latex extracts. Our results clearly indicate that cross-reactivity determinants exist between 30- and 42-kD allergens of Indian jujube and the identified 42-kD allergen of latex, and add to findings regarding the 'latex-fruit syndrome' described in the literature. Further study of the cross-reacting allergens identified in Indian jujube may help to elucidate the mechanisms underlying this syndrome.

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