Clinical and Molecular Allergy

BioMed Central

Research

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The human allergens of mesquite (*Prosopis juliflora*)

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Published: 05 July 2004

Clinical and Molecular Allergy 2004, **2**:8 doi:10.1186/1476-7961-2-8

Received: 23 April 2004

Accepted: 05 July 2004

This article is available from: http://www.clinicalmolecularallergy.com/content/2/1/8

Abstract

Background: A computerized statistical analysis of allergy skin test results correlating patient reactivities initiated our interest in the cross-reactive allergens of mesquite tree pollen. In-vitro testing with mesquite-sensitized rabbits and a variety of deciduous tree pollens revealed so many cross-reactivities that it became apparent there could be more allergens in mesquite than previously described in the world literature. Our purpose was to examine the allergens of mesquite tree pollen (*Prosopis juliflora*) which elicit an IgE response in allergic humans so that future research could determine if these human allergens cross-react with various tree pollens in the same manner as did the mesquite antiserum from sensitized rabbits.

Methods: Proteins from commercial mesquite tree pollen were separated by polyacrylamide gel electrophoresis in the presence of sodium-dodecyl-sulphate. These mesquite proteins were subjected to Western blotting using pooled sera from ten mesquite-sensitive patients and goat anti-human IgE. The allergens were detected using an Amplified Opti-4-CN kit, scanned, and then interpreted by Gel-Pro software.

Results: Thirteen human allergens of mesquite pollen were detected in this study.

Conclusion: The number of allergens in this study of mesquite exceeded the number identified previously in the literature. With the increased exposure to mesquite through its use in "greening the desert", increased travel to desert areas and exposure to mesquite in cooking smoke, the possible clinical significance of these allergens and their suggested cross-reactivity with other tree pollens merit further study.

Background

Mesquite (*Prosopis juliflora*) is a major cause of allergic disease in the southwestern United States [1,2], Mexico [3], Saudi Arabia, South Africa [4,5], Kuwait [6], United Arab Emirates (UAE) [7], and India [8]. *Prosopis juliflora* is a legume with several variations [2] that has been used for the reclamation of desert lands and as a wood resource [4,8], with the end result that its easily dispersed and its far-traveling pollen [1,4,8] is an abundant and significant source of allergens [5]. Novey [1] reported that mesquite was the most prevalent pollen sensitizing 100 of his patients in a California study, while Bener et al. [7], report that 45% of the patients they tested in the UAE were sensitive to *Prosopis*. In addition to pollen exposure, the burning of mesquite wood and its resulting smoke may be another source of exposure to some of these same allergens [9,10].

Our interest in mesquite was initiated by a clinician who observed that many of his allergy patients (1598 out of

4361 patient cases) were sensitized to mesquite pollen even though most of them had no known direct exposure to mesquite (Personal communication with Allan D. Lieberman, M.D., The Center for Occupational and Environmental Medicine, 7510 Northforest Dr., N. Charleston, SC, USA). A computerized-analysis of skin test data from this medical practice showed mesquite correlating with a high number of other seemingly unrelated commercial antigens, leading us to believe it might have a proclivity for cross-reactivity [11]. Tree pollen cross-reactivity with mesquite was confirmed using rabbit antiserum for both Ouchterlony (unpublished study) testing and Western blotting [12], but the relevance to human allergy needed to be established. The aim of the current study was to amplify the present knowledge of mesquite allergens which affect humans preliminary to investigating the impact of mesquite pollen cross-reactivity.

Methods

Proteins from a 1:2 dilution of mesquite tree pollen extract (*Prosopis juliflora* var. glandulosa)(Greer Laboratories, Inc., Lenoir, North Carolina) in Laemmli Sample buffer (Bio-Rad) were separated by polyacrylamide gel electrophoresis in the presence of sodium-dodecyl-sulphate (SDS-PAGE) according to the method of Laemmli [13]. The protein concentration of the mesquite extract was as determined by Greer Laboratories using the Kjeldhal Method [14]. Six lanes on each of 4 replicate gels were loaded with 8 μg of mesquite extract protein. A Mini-Protein 3 Electrophoresis Apparatus (Bio-Rad, Hercules, California) and a pH 8.3 Tris-glycine buffer were used. Polyacrylamide concentration of the precast 10 lane gels was 4% for the stacking gel and 12% for the resolving gel. Electrophoretic migration was performed at 21°C and 150 v constant voltage for 35 minutes until the bands migrated to the lower edge of the gel. Protein bands were visualized by Brilliant Blue R-250 (Fisher Biotechnology, Inc., Fair Lawn, NY) staining. Dual Color Precision Plus Protein Standards expressing calibration points of 10, 15, 20, 25, 37, 50, 75, 100, 150 and 250 kd (Bio-Rad) were included in the electrophoretic separation. Protein bands were destained and scanned at 150 pixels per inch (ppi) setting by a Hewlett Packard ScanJet 4100C and interpreted using Gel-Pro 3.1 computer software (Media Cybernetics, Silver Spring, Maryland) to determine their molecular weights, relative optical densities and approximate protein distribution.

Immunoblotting was performed using a Mini-Trans-Blot Electrophoretic Transfer Cell (Bio-Rad) according to the method of Towbin et al. [15]. Unstained mesquite pollen extract (Greer Laboratories) electrophoresis gels were electroblotted onto 0.2 μm Trans-Blot Transfer Media (nitrocellulose membranes) (Bio-Rad). Membranes were blocked with 5% blotting grade nonfat milk (Bio-Rad) in wash buffer of PBST (phosphate buffered saline: 10 nM sodium phosphate, 150 nM sodium chloride and 1% Tween-20 (Bio-Rad).

Sera from fifteen mesquite-positive patient donors were tested for their IgE responses using this electrophoresis and Western blotting protocol and all produced positive IgE responses to mesquite pollen allergens. Ten of these patients were then chosen to participate in the study because they not only tested mesquite-positive but also provided at least 250 ml of their sera, the minimum need for sequential experiments. The five patients who were not included in the study provided insufficient quantities of sera for the present experimental replicates and future studies. These sera were obtained from both commercial sources and private physicians (Table 1). Each donor was given a research number to protect his/her confidentiality and each donor from a private physician's practice signed a consent form.

The pooled sera from these 10 patients were placed in a 55°C water bath for 30 minutes to destroy complement. These pooled sera were diluted 1:2 in the wash buffer with 0.05% blotting grade nonfat milk and incubated with the nitrocellulose blot overnight. Bound IgE was detected by incubating the blot for 5 hours, using a 1:1000 dilution of goat anti-human IgE conjugated to peroxidase (Sigma, Saint Louis, Missouri). An Amplified Opti-4-CN Kit (Bio-Rad) was used for visualization of the IgE responses. Between each step the nitrocellulose blots were washed three times for five minute periods with PBST. When dry, the blots were scanned into the computer at both 150 and 1200 ppi and interpreted by Gel-Pro 3.1 software, as were the electrophoresis gels

Human serum, determined by the supplier to be non-allergenic by the Pharmacia Method, was purchased for use as a negative control (PlasmaLab International, Everett, Washington). Six mesquite lanes on three replicate blots were incubated with the non-allergic serum and goat anti-human IgE as previously described, providing negative controls.

As a separate control for non-specific background, mesquite antigen was subjected to electrophoresis and blotted and detected as previously described except for the omission of the human sera.

Results

Fourteen mesquite protein bands, visualized by SDS-PAGE using Brilliant Blue-250 dye, were detected by Gel Pro 3.1 software (Figure 1). The molecular weights of these gel bands were 11, 14, 16, 17, 18, 19, 20, 27, 30, 36, 44, 56, 71 and 99 kd. The 19 kd band, detected by Gel-Pro 3.1 was not visible to the naked eye. Twelve of these gel

Table 1: Sources of allergic sera and testing methods determining mesquite sensitivity.

	Sources of Mesquite-Allergic Sera	Diagnostic Testing of Donors	Donor Sensitivities to Other Allergens
1.	PlasmaLab International, Everett, WA	Pharmacia CAP Method : IgE Class 4 response* (very high) of 20.4 kU/L specific to mesquite pollen	pollens (trees, grass and weeds), molds, epidermals, and foods
2.	PlasmaLab International, Everett, WA	Pharmacia CAP Method : IgE Class 3 response (high) of 17.0 kU/L (kilo units per liter) specific to mesquite pollen	pollens (trees and weeds), molds, epidermals and foods
3.	PlasmaLab International, Everett, WA	Pharmacia CAP Method : IgE Class 3 response (high) of 12.4 kU/L specific to mesquite pollen	pollens (trees, grass and weeds), molds, epidermals and foods
4.	Cliniqa Corporation, Fallbrook, CA	RAST Testing: Class 4 (501–1500 SIE units using the THABEST IgE Scoring System)**	pollens (trees, grass and weeds), molds, epidermals and foods
5.	Cliniqa Corportion, Fallbrook, CA	RAST Testing: Class 3 (151–500 SIE units using the THABEST IgE Scoring System)	pollens (trees, grass and weeds), molds, epidermals and foods
6.	Private Physician, Scottsdale, AZ	Positive scratch test using mesquite antigen from Greer Laboratories, Inc.	pollens and molds
7.	Private Physician, Winchester, VA	Positive (severely allergic) intradermal skin test using mesquite antigen from Greer Laboratories, Inc.	no other known allergies
8.	Private Physician, Winchester, VA	Positive (moderately allergic) intradermal skin test using mesquite antigen from Greer Laboratories, Inc.	milk allergy; no other known allergies
9.	Private Physician, Winchester, VA	Positive (moderately allergic) intradermal skin test using mesquite antigen from Greer Laboratories, Inc.	pollens (trees, grass and weeds), molds, epidermals and foods
10.	Seraplex, Inc., Duarte, CA	Pharmacia CAP Method Class 3 response (high) of 12.5 kU/L specific to mesquite pollen.	pollens (types unavailable) and foods

*Obtained from the class description in the package insert of Pharmacia CAP Method. **Obtained from the class description on patient records, Cliniqa, Inc.

bands produced an IgE response when subjected to Western blotting (Figure 1). Gel-Pro 3.1 software interpreted these blot bands as 11, 16, 17, 18, 20, 27, 30, 36, 44, 56, 71, and 99 kd. An additional IgE response by a 64 kd band not present on the gel was detected on the blot resulting in thirteen total blot bands (Figure 1). The remaining 14 kd gel band produced only a slight image when blotted.

The total protein loaded into each lane of the gel was 8.0 μg but only 6.3 μg total protein was detected by Gel-Pro software in each lane. The protein in each band varied from 0.22 μg to 0.70 μg with the average being 0.44 μg.

The control blot using the same mesquite antigen and purchased non-allergic human serum (PlasmaLab International, Everett, WA) showed no binding at all when blotted. The control for non-specific background produced a faint set of bands in the 18 to 20 kd area. The optical densities of the non-specific background in the control blots were 30 times less intense than the test band of 18 kd and 89 times less intense than the test band of 20 kd.

Discussion

Literature describing the allergens of *Prosopis* spp. is sparse, with the majority of published research coming from Thakur and fellow scientists in India. Using gel filtration chromatography and polyacrylamide gel electrophoresis with 7.5% gels, Thakur and Sharma separated *Prosopis juliflora* into six fractions of 13, 20, 27.5, 41, 55.5 and 81 kd [16]. In a guinea pig skin prick test using each of these fractions, they found the 20 kd fraction had major allergenic activity [16]. Using sensitized rabbits, Thakur [8] found that the 10 and 20 kd fractions were both glycoproteins. Thakur reported a 45% success rate in using both the mesquite crude allergen extract and the 20 kd glycoprotein fraction in human desensitization to *Prosopis*.

More et al. [9] used 10–20% gels to investigate the allergens of mesquite in Arizona, USA, and reported IgE responses to 59 and 66 kd proteins in the pollen, wood and wood smoke of mesquite. They commented that more allergens of mesquite pollen were present but did not give further descriptions [9].

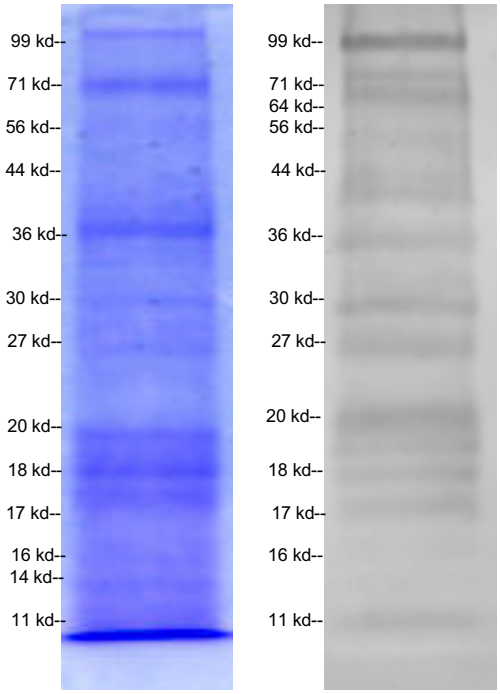

Figure 1
Electrophoresis gel lane of mesquite antigen (left) and mesqite allergens demonstrated by Western blotting (right). Pooled allergic human serum produced thirteen IgE responses to mesquite tree pollen (*Prosopis juliflora* var. glandulosa) when tested by Western blotting. The molecular weights of these allergens ranged from 11 to 99 kd when they were blotted from a 12% electrophoresis gel onto nitrocellulose as detected by the Amplified Opti-4-CN system (Bio-Rad).

In comparing our results (using 12% gels and pooled human sera for Western blotting) to Thakur's research, we concur with the 20 kd band. The non-specific background in the 20 kd area detected in our negative controls appeared negligible in this study since the optical intensity of the 20 kd band was 89 times stronger than the control.

It is possible that these bands of the present research and Thakur's [17] (in parentheses) are the same bands: 11 kd (13 kd), 27 kd (27.5 kd), 44 kd (41 kd), 56 kD (55 kd), 71 and 99 kd (81 kd). Discrepancies could be due to the differences in pollen extracts, the concentrations of the gels, the sera used [18], the accuracy of molecular weight standards or the sensitivity of the detection systems.

In addition to the bands described by Thakur, we found bands at 17, 18, 36 and 64 kd.

The strong 64 kd band present on the blot but not on the gel could be explained by the sensitivities of the detection systems of the gel (500 ng) and the blot (5 pg), differing by a magnitude of 1000. A faint 19 kd band was visible on the blot and on the gel but we did not list it as an separate allergen because it was not distinct from the 20 kd band. This could have been the non-specific background detected in our negative controls. Gel band 14 blotted only faintly and was not included in the total numbers of mesquite allergens in this study.

Before pooling the sera we noticed variation in the reactivity patterns of several of the 10 serum donors at the 56 – 65 levels. When pooled this seemed to result in a blurred area between 56 and 65 kd whereas in individual donors there were clear bands present.

Some donors' sera were missing a band present in other donors. It is possible patients simply have different reactivity patterns to the same pollen or this could be caused by their exposures to variations in *Prosopis* pollen species. Varieties of *Prosopis juliflora* in the southwest United States include – glandulosa, Torreyana, and velutina [2]. *Prosopis* species are morphologically variable, are considered a synagameon (habitual hybridization), and these hybrids are fertile [3]. Patient exposure to mesquite varieties or hybrids could result in different IgE banding patterns, especially when comparing worldwide distributions of *Prosopis*.

Mesquite (*Prosopis juliflora)* is considered a serious allergen. Exposure to this pollen in arid areas (both naturally occurring and by intentional plantings), through international travel and military deployment is significant. In addition to pollen exposure, mesquite smoked foods are popular and exposure to mesquite antigens may occur both in food preparation and consumption.

The relevence of cross-reactive mesquite allergens to humans needs serious consideration. The human allergens of mesquite visualized in this study compare favorably to the cross-reactive mesquite allergens we previously described [12] using mesquite-sensitized rabbits. While this does not necessarily mean that the human allergens to mesquite are cross-reactive, it does suggest the possibility.

Conclusion

This research suggests that there are at least thirteen human allergens in mesquite tree pollen. The significance of the human allergens of mesquite and their possible cross-reactivities with other tree pollens, as suggested by a

previous mesquite-sensitized rabbit cross-reactivity study, merit further research.

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