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Major allergens of date palm (*Phoenix dactylifera* L.) pollen

Identification of IgE-binding components by ELISA and immunoblot analysis

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The IgE-binding components of date palm (*Phoenix dactylifera* L.) pollen were determined by ELISA and Western blotting in atopic patients in order to identify its major allergens. From a pool of previously identified allergenic fractions and sera from 15 skin-test-positive, atopic subjects, four components of 12, 14.4, 57, and 65–67 kDa were found to bind IgE in 80–93% of sera. Two other components of molecular masses 28–30 and 37–40 kDa also bound 60–80% of atopic sera. The immunologic specificity of date-pollen allergen that induced antibody response in sera of atopic patients was confirmed with ELISA. Furthermore, most of the reactivity in pooled positive atopic serum and antiserum raised in rabbits was eliminated after the sera were absorbed with the allergen. IgG immunoblot analyses showed varying degrees of cross-reactivity with common local allergens, notably Bermuda grass, but were generally of low intensity. These results indicate that date pollen has six major allergens with the 12, 14.4, 57, and 65–67 kDa bands binding 80–93%, and the 28–30 and 37–40 kDa bands 60–80% of atopic sera. We propose that these major allergens be assigned the notations "*Pho d* I" to "*Pho d* VI" in the order listed.

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Date-palm pollen has recently been identified as one of the major causes of allergic diseases within an atopic population in Riyadh, Saudi Arabia, with 25% of the patients studied giving positive skin test reactions to crude pollen extract as well as some of its Sephacryl S-200 fractions (5, 8). When the protein components of the crude extract were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and analyzed by Western blotting, 12 IgE-binding components were identified in immunoblots with pooled positive atopic sera, and a total of 18 IgE-binding components in immunoblots with sera from individual patients and date-palm pollinators. Furthermore, when peripheral blood lymphocytes (PBL) from atopic, date-pollen-sensitive patients were stimulated with either the crude pollen extract or its fractions, or phytohemagglutinin (PHA) or interleukin-2 (IL-2), they showed significantly enhanced proliferative response and cytokine (IL-2 and IL-4) production, as compared with normal PBL. These results indicate that this pollen has an *in vivo* role in IgE induction, emphasizing the importance of date pollen as a potent allergen (8).

The aim of this study was to use ELISA and IgE immunoblot analyses to identify the principal IgE antiallergens in sera from the atopic population that reacted to the pollen extract and its fractions in preliminary studies. For these studies, the most allergenic fractions were pooled and used as a capture antigen for date-pollen-specific antibodies in individual sera and also to determine the suitability of this allergen for serodiagnosis and probable therapy. An attempt was also made to identify any relationships existing between the IgE- and IgG-reactive bands. Since most of the patients studied have multiple skin test reactivities to other pollen allergens, and cross-reactivities of date pollen with these pollen allergens have not been investigated, an assessment of date-pollen components cross-reactive with other allergens was made. Bermuda grass, the most common allergen found in Saudi Arabia (17, 20, 21),

and other allergens to which patients attending our local clinic commonly react were investigated.

Material and methods

Date-pollen allergen

IgE-reactive fractions "B", "C", "D", and "G" from Sephacryl S-200 size exclusion chromatography (5) were pooled and concentrated to 500 µg/ml protein. The final pool was evaluated in experiments to make sure that it was as antigenic and allergenic as the original whole extract and its fractions.

Human sera

Sera from 60 atopic patients attending our allergy clinic were collected. Most of these patients had high total serum IgE levels and suffered from asthma, rhinitis, or at least one allergy, including 15 with positive skin test to date pollen. Cord serum and sera from 10 young, normal, healthy blood donors were also collected and stored at −20°C until required for various assays.

Estimation of total IgE

The 15 atopic, skin-test-positive sera and 10 normal sera were assayed for their total IgE content by the Phadezym IgE PRIST (Pharmacia Diagnostics AB, Uppsala, Sweden) method, according to the manufacturer's directions. The individual values for each serum as well as the group means for the atopic, date-pollen-allergic patients and the normal control group were computed and analyzed.

SDS–PAGE and immunoblotting

The discontinuous method of Laemmli (9) was used for SDS–PAGE as previously described, the only difference being that 13.5% separating gels were used instead of 12% gels because the 13.5% gels helped to resolve some low-mol.-wt. protein bands which otherwise remained at the dye front. All subsequent procedures were performed exactly as described earlier (5, 13).

Radioiodination of anti-human IgE

The chloramine-T catalyzed method described by Langone (10) was followed. Briefly, 1 mg/ml antihuman IgE (Dako, Denmark) was radioiodinated by chloramine-T (2.25 mg/ml), sodium metabisulfite (7 mg/ml), and 0.5 mCi NaI (^{125}I) (Amersham, UK). After the completion of the iodination reaction and elution on Sephadex G-25 column, radioactivity in the fractions was determined (LKB Wallac 1272 Clinigamma, Turku, Finland), and the specific activity was determined from the fractions of the first peak containing the labeled goat antihuman IgE.

Immunodetection and autoradiography

All human sera for immunodetection by autoradiography were diluted 1/20 (v/v) in PBST/1% BSA, but those for enzymatic detection were diluted 1/10. For the detection of IgE-staining immune bands, radioiodinated, antihuman IgE and goat antihuman IgE alkaline phosphatase labeled conjugate (Binding Site, Birmingham, UK) were used. All alkaline phosphatase reactions were performed as previously described (5, 13). Immunostaining with radiolabeled antibodies was performed as detailed below. IgE bands were detected by incubating nitrocellulose strips in ^{125}I antihuman IgE (20000 cpm/strip, specific activity = 5.04 MBq/µg) overnight. Either IgG bands were detected by alkaline phosphatase labeled conjugated antihuman IgG as described (5), or the strips were allowed to react for 2 h with protein G labeled ^{125}I (20000 cpm/strip, Amersham, UK). After washing (2 × 30 min in TTBS/1% BSA), strips were air-dried at room temperature and loaded into Kodak X-Omatic cassettes with regular intensifying screens using XAR-5 X-OMAT AR diagnostic films (Eastman Kodak, Rochester, NY, USA). Cassettes were stored at −70°C for 6–14 days. Films were developed, and those with optimum exposure quality were analyzed visually and with the aid of a Bio-Rad Video Densitometer (Model No. 620, Bio-Rad, Richmond, CA, USA) interfaced with an IBM AT personal computer. All membranes and autoradiographs were photographed and kept as a permanent record.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed by the indirect method of Voller et al. (23) as previously described (5), the only difference being that plates were coated with purer allergen pool because of its high potency relative to the whole extract (5, 8). The mean and SD for the control and atopic sera were compared, and the results analyzed as reported (5).

Absorption tests

Rabbit antiserum, pooled positive human sera, preimmune rabbit serum, and pooled control sera were used in absorption experiments with the allergenic fraction by the method described by Parsons et al. (12). The absorbed sera were tested in ELISA as described above and in inhibition experiments to assess the specificity of the ELISA as well as the suitability of the reagents for serodiagnosis.

Cross-reactivity studies

Most of the atopic patients demonstrated multiple skin test reactivities to other allergens; therefore, local and commercial extracts of the most common allergen, Bermuda grass (18, 20–22), as well as of *Artemisia*, rye, timothy, and acacia from either Hollister-Stier (Miles, Inc., Elkhart, IN, USA) or Greer Laboratories, Inc. (Lenoir, NC, USA), were used in immunoblots to assess any possible cross-reactions that date pollen might have with these allergens. The individual allergens were separated on the same SDS–PAGE gel by a multichannel comb and the separated components transferred onto nitrocellulose membrane. Polyspecific date-pollen antiserum raised in rabbits (5) was used as primary antibody, since atopic sera with monospecific reactivity to the individual allergens were not available. Immunodetection and color development steps were performed as described before (5, 13).

Results

The results of SDS–PAGE with 13.5% gel (Fig. 1) helped to reveal additional date-pollen components of lower molecular mass (8–12 kDa) than those previously reported, and some bands above 94 kDa were also seen.

Total IgE values in the individual sera (results not presented) were found to be higher in 13 of the 15 patients than those considered to be normal for the Saudi population (1). The overall patient mean was significantly higher than the mean of the control sera ($P = 0.0021$).

Immunoblot results using the 15 sera from atopic, date-pollen-sensitive patients showed that a total of 16 different SDS–PAGE-separated components bound IgE. The antiserum-binding patterns were heterogeneous for the different sera, but the binding patterns appeared to be consistent for the individual sera from one experiment to another. Autoradiography appeared to detect fewer bands than enzyme conjugate probed immunoblots for any particular serum investigated. This could be related to residual alkaline phosphatase present in human serum. On some of the enzyme conjugate probed membranes, some bands were discrete, but others were faint and faded very quickly even before they could be photographed, while on some autoradiographs some bands appeared to be fused together as single bands, making it difficult to identify and estimate their molecular mass accurately.

Typical enzyme-stained bands and autoradiographs of the IgE-reactive bands in the different sera are shown in Fig. 2a(i) and (ii), respectively, and diagrammatically in Fig. 2b. A summary of the date-pollen-specific IgG and IgE antibodies and their interrelationship is presented as a frequency histogram in Fig. 3. It can be seen from the histogram that all the atopic patients' sera bound IgG at a wide range of molecular mass (8–94 kDa and above), while the IgE bands were reactive with peptides only at certain molecular mass ranges.

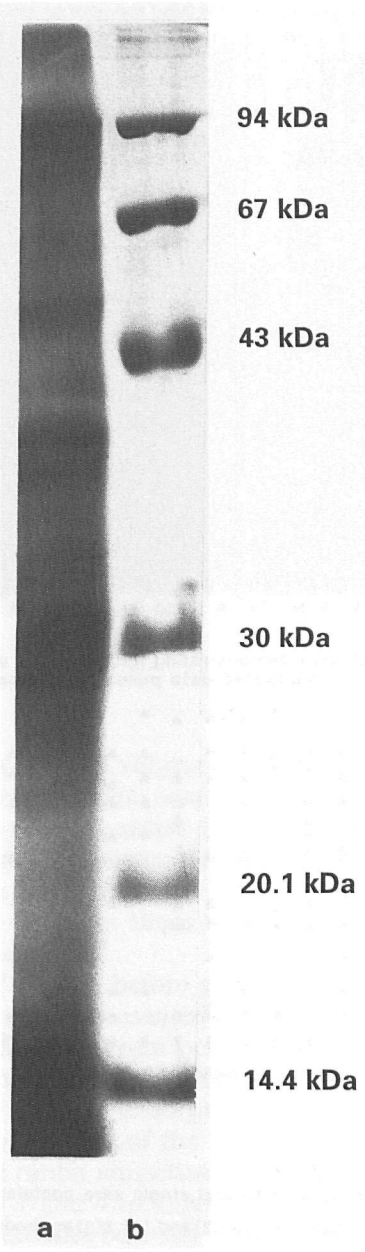
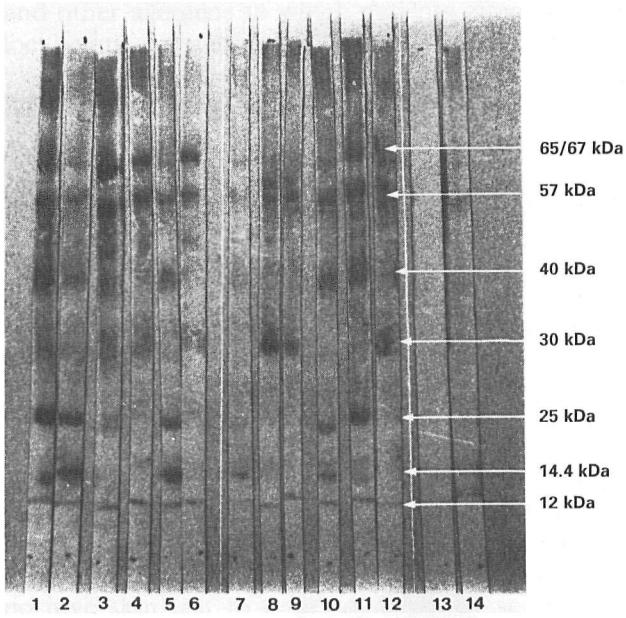
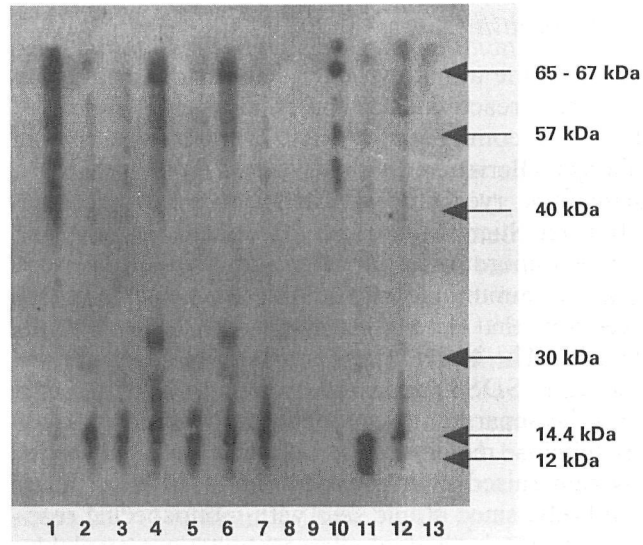


Fig. 1. SDS–PAGE of date-pollen allergen and molecular mass markers separated on 13.5% gel and stained with Coomassie brilliant blue. Column a shows date-pollen allergen components ranging in molecular mass from 8 kDa to over 94 kDa. Column b shows low molecular mass markers (Pharmacia): phosphorylase-b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), and lactalbumin (14.4 kDa).

Detailed analysis of the banding patterns indicated that four components with molecular mass of





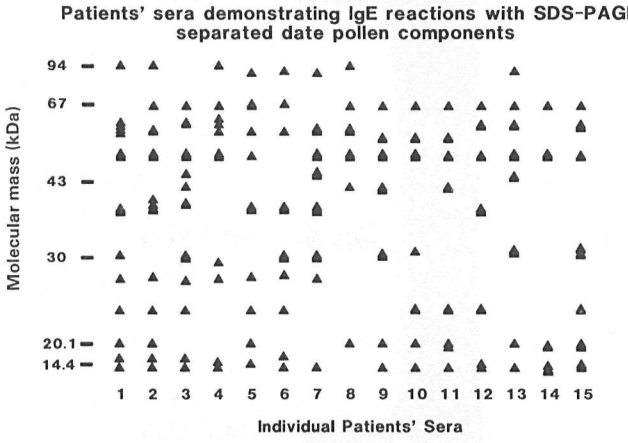


Fig. 2. a) Typical IgE immunoblots of some individual atopic sera immunostained with (i) alkaline phosphatase labeled goat antihuman IgE conjugate and (ii) radioiodinated goat antihuman IgE (specific activity = 5.04 MBq/μg). These are representative of results from different experiments. Strip 13 in (i) is cord serum control, and strips 8 and 9 in (ii) are cord serum and normal serum pool, respectively. Note that strips in (i) do not appear to be properly aligned because of heat produced during photography which caused nitrocellulose strips to crinkle. b) Diagrammatic representation of 15 patients' sera demonstrating IgE antibody reactions with date-pollen allergen. Results were abstracted from several blots of radioiodinated as well as enzyme-stained strips (as in a). Bands which faded before photography were also noted and included.

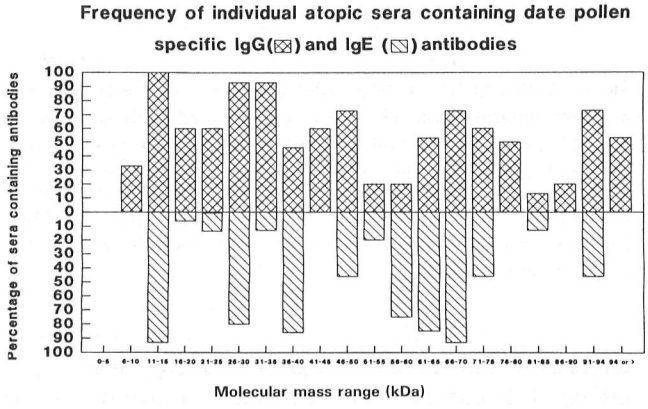


Fig. 3. Frequency histogram of atopic sera demonstrating IgG/IgE antibody relationship to each of the SDS–PAGE-separated date-pollen components. Results were collated from experiments in which radioiodination and enzyme staining were used to detect Western-blotted pollen components. The number of sera that bound components at each molecular mass was calculated as percentage of total number of sera.

12, 14.4, 57, and 65–67 kDa bound from 80 to over 90% of sera, and two others of 28–30 and 37–40 kDa bound 60–80% of the atopic sera. Densitometric analyses on a Bio-Rad Video Densitometer (model no. 620; results not shown) of the intensities

Table 1. IgE and IgG ELISA of 60 atopic and 10 control sera. Positive and negative values were obtained with an IBM AT PC programmed to calculate mean and standard deviation of test and control sera. Values were classified on the scale 0–4, and values 2 and above were considered to be positive

	Positive	Negative
IgE ELISA		
Atopic (+ve) (n=15)	15	0
Atopic (−ve) (n=45)	0	45
Normals (n=10)	0	10
IgG ELISA		
Atopic (+ve) (n=15)	15	0
Atopic (−ve) (n=45)	5	40
Normals (n=10)	0	10

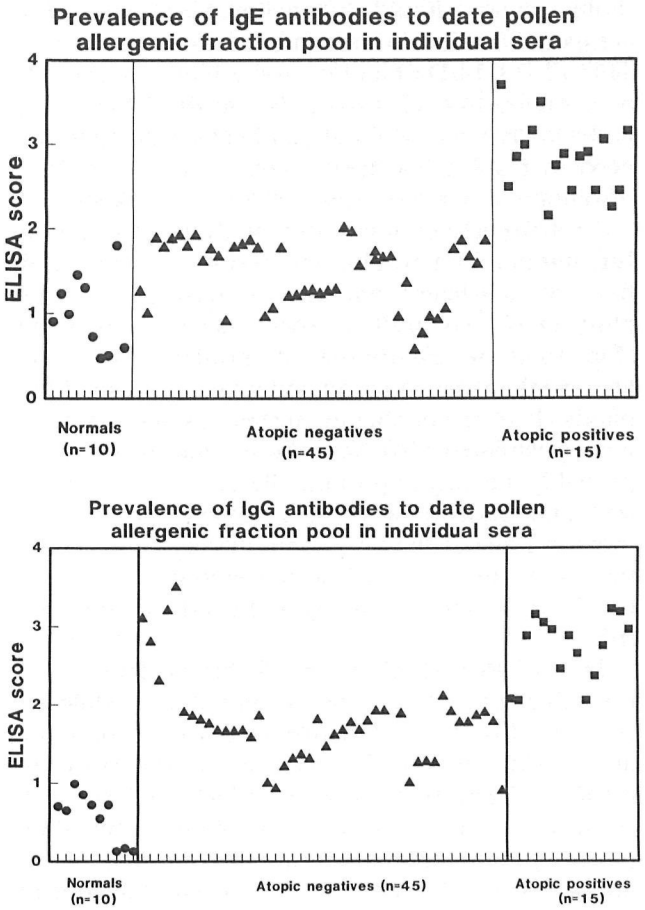


Fig. 4. a) IgE ELISA distribution of individual sera grouped as normal, atopic negative, and atopic positive. b) IgG ELISA distribution of individual sera grouped as normal, atopic negative, and atopic positive.

of the various bands on the autoradiographs indicated that the 12, 14.4, and 65–67 kDa bands were always the most intense bands in 50–73% of sera. In some autoradiography experiments, some bands appeared to be single fused bands, making actual molecular mass estimations difficult.

ELISA results are presented in Table 1 and in Figs. 4a and b. When the allergenic fraction pool was used as capture antigen, some putatively negative sera (five of 45 subjects who were skin-test-negative) gave positive results to IgG antibodies, while all the positive atopic sera reacted positively. None of the normal control sera gave positive results in these tests. In IgE ELISA tests, only the positive sera reacted as such; there were no "unexpected" negative results.

Table 2. ELISA score of different sera before and after absorption with date-pollen allergen. Polyspecific rabbit immune serum and pooled atopic positive human serum lost antibody reactivity after absorption to the values of control sera

Serum	ELISA Score	
	Unabsorbed	Absorbed
Rabbit preimmune serum	1	1
Rabbit antiserum	4	1
Human positive serum	3	1
Normal human serum	1	1

Table 3. Cross-reactivities between date pollen and other allergens as determined by immunoblotting of SDS–PAGE of the various allergens by polyspecific date-pollen antiserum raised in rabbits. L=locally extracted allergens; C=commercially produced allergens

Molecular mass (kDa)	Allergens							
	Artemisia		Rye	Timothy		Acacia	Bermuda grass	
	L	C	C	L	C	C	L	C
94 or >	+			+	+	+	+	+
92–94				+	+	+	+	+
88–89		+	+	+	+	+	+	+
83–84		+		+	+	+	+	+
76–80			+	+	+	+	+	+
55–60							++	++
49–55							+	+
34–45			+	+	+			
28–32	+							
Total**	2	2	3	6	6	5	7	7

** =total number of cross-reactive bands.
+ =weak reaction ++=strong reaction.

Results of ELISA before and after absorption of the various sera are presented in Table 2. Apart from the normal human serum pool and the preimmune rabbit serum negative control sera, which did not react before or after absorption, there was appreciable loss of activity of the human positive serum pool and the rabbit antiserum (ELISA score reductions of 3 to 1 and 4 to 1, respectively).

Cross-reactivities between the various allergen extracts are summarized in Table 3. From one to seven cross-reacting IgG bands (generally faint) were identified for the various locally extracted allergens as well as those purchased from commercial firms. Local *Artemisia* extract cross-reacted with date-pollen components in the 28–32 kDa range. Rye and timothy grass cross-reacted with a fused band between 34 and 42 kDa, and both the local and commercial allergen extracts of Bermuda grass cross-reacted with seven components of date pollen, with a slightly more intensely stained but fused broad band between 55 and 65 kDa. Timothy grass, acacia, and Bermuda grass demonstrated between five and seven cross-reactive bands. Apart from the Bermuda grass cross-reactive band between 50 and 65 kDa, all the cross-reactivities observed were faintly stained and faded quickly.

Discussion

Allergenic fractions identified in preliminary studies (5, 8) were chosen for the present study and reassessed for their potencies as observed in previous (5) as well as in the new experiments being investigated. The allergenic pool used in this study was reliable for the reproducibility of all results reported here. A higher percentage gel was used in this work with the aim of resolving lower molecular mass bands which otherwise remained at the dye front and appeared to have a molecular mass of 14.4 kDa in 12% SDS–PAGE gels. Indeed, not only was this aim achieved, but the purer nature of the antigen pool used in the present study helped also to identify some higher molecular mass bands of over 94 kDa. These findings have revealed a previously unrecognized complexity of date-pollen components and serves to warn researchers in this field that a careful choice of SDS–PAGE gel concentrations is essential for accurate estimates of the number of antigenic and allergenic peptides in any extract (4, 18, 22).

The mean of the total IgE values in the atopic-positive patients' sera was significantly higher than the normal control group ($P = 0.0021$). These results agree with other studies (1, 3, 11, 15).

All the skin-test-positive sera demonstrated varying degrees of date-pollen-allergen specific IgE reactivity in both ELISA and immunoblot experiments. The results of IgE immunoblot patterns showed as much heterogeneity as that reported in similar studies for other allergens (4, 7, 14, 18, 22). Most sera bound six main allergen bands, a binding frequency which is higher than those found for other allergens (4, 7, 14, 18, 19), but this may be due to the fact that some of these bands are subunits of some main bands of the same allergen, because autoradiography, which appears to be more specific, detected fewer bands, a point that has been postulated for the 65–67 kDa peptide (5) and needs to be further investigated. The components that were recognized by 80–93% of patients' sera (the 12, 14.4, 57, and 65–67 kDa bands) can be considered to be the most frequent, and those that bound 60–80% of patients' sera (the 28–30 and 37–40 kDa bands) to be the least frequent major allergens. It appears, however, that these allergens have to be repeatedly separated to reach purity, and to be assessed in a larger sample of allergic patients before their individual allergenic functions can be well defined.

It is noteworthy, however, that the 14.4-kDa band, which was seen in all fractions A–I during the previous studies (5) as a single band, but which was intensely stained only in fraction "G", probably owed its intensity to the aggregation of some lower bands as well as the 12- and 14.4-kDa bands. Fraction "G", as indicated by size exclusion chromatography kinetics, should contain lower molecular-mass components than do the other fractions in the pool. This 12–14.4-kDa band on some blots appeared to be a single, 14.4-kDa band, but most of the time it had a lower band of about 12 kDa as well; hence the need to resolve the bands lower than 14.4 kDa, a resolution which has been achieved in this study.

A retrospective comparative analysis of the present IgE immunoblot results and results of IgG bands from the gardeners and the two groups of atopic patients (5), as well as the frequency histogram (Fig. 3), shows clearly that the principal IgE bands are also the most common and intensely stained IgG bands. It is reasonable to suggest, as some authors have postulated (16), that these main allergens are probably the only important allergens of date pollen, and that patients who have certain alleles at the preferred loci of the histocompatibility (HLA) complex and are atopic allow the epitope recognition that leads to specific allergy in the individual patient (6).

IgG ELISA (Fig. 4b and Table 1) detected an extra five out of 45 skin-test-negative sera, while IgE ELISA (Fig. 4a and Table 1) detected only the atopic, skin-test-positive sera and no others. These results indicate that the IgG ELISA analyses were sensitive enough to detect traces of circulating date-pollen-specific IgG antibodies in the sera of five atopic, skin-test-negative subjects who had not regressed to an allergic state (2). The indication is that this method could be used to study the etiology of date-palm pollinosis, and its clearance in human serum, and to diagnose asymptomatic subjects. It is encouraging to note that IgE ELISA (Fig. 4a and Table 1) appears to be able to select atopic, date-pollen-positive sera with precision. This is consistent with previous results in which crude whole pollen extract and fewer patients' sera were used (5). The hypothesis that the ELISA results are reliable has to be proved in detailed comparison with further immunoblots, RAST, and conjunctival and intradermal challenges as well as bronchial provocation tests before it can be considered to be unequivocal. The fact that antibody reactivity could be eliminated after absorption experiments is also encouraging (Table 2), thereby supporting the notion that the allergen is specific enough to be standardized for serologic screening and possible treatment of date-palm pollinosis.

Cross-reactivities among common local pollen antigens from plants that are botanically unrelated to the date palm were observed on IgG blots with polyspecific antiserum raised in rabbits (Table 3). However, all but one of the cross-reacting bands (that with a broad 50–65 kDa band in Bermuda grass) were faint and faded quickly, indicating that these are reactions of weak recognition. Interest-

ingly, some of the major allergens of Bermuda grass (4, 18, 22), as well as two of the major allergens of date pollen, bind within the same molecular mass range. These are IgG immunoblots and do not necessarily reflect allergenic cross-reactivity; however, it is important to ascertain by anti-IgE immunoblots using atopic sera whether there are any epitope similarities between Bermuda-grass and date-pollen allergen extracts. This is a topic that will have to be addressed in further investigations. The locally extracted *Artemisia* and the rye and timothy purchased from commercial firms cross-reacted with date-pollen components between 34 and 45 kDa, and in the 28–32 kDa range, and all of these should be further studied in detail in IgE blots with individual atopic sera.

It must be remembered that the antiserum used was of a polyspecific nature, and this could account for several weak cross-reactions which might not have been seen if monospecific serum or sera from patients with singular allergic response to each of the various allergens had been used. Cross-reactivities like those noted in this work can be explained in one of two ways; different populations of antibodies can recognize the proteins from the different sources, or the different sources of proteins can share common structural determinants which the same IgG antibodies bind. There is also the remote possibility, that these weak IgG reactions were due to antibodies produced against some constituents in the feed of the rabbits or other allergens within the general animal facility environment, and were not specific to date-pollen allergens.

In patients with multiple allergies, antibodies directed against the same or similar determinants in the different allergens could act in concert towards the sensitization of the patient. Detailed comparative studies of all the immunoglobulin classes and the various IgG subclasses in relation to each of the major date-pollen allergens by the use of sera from different groups of patients and exposed persons would help clarify the etiology of date pollinosis.

Dates are one of the most important fruits eaten throughout Saudi Arabia, the Gulf, and the Mediterranean countries, and they are, to a lesser extent, eaten on special occasions in various other parts of the world. Thus, it is imperative that any potential allergies or pollinosis that their consumption could cause be carefully evaluated and understood. Furthermore, there are several cultivars of the date palm, and this means obvious ecocline and ecotype differences which could be a source of variation in pollen extracts from different palms and from different locations. Since the extract used in this work was prepared from pollen collected from over 50 male plants comprising a multiplicity of cultivars, our findings can be considered to be representative of common cultivars grown in the Riyadh area of Saudi Arabia. Attention, however, needs to be focused on any varietal differences in later investigations, so that standardized allergenic extracts from the relevant subspecies or cultivars can be prepared for routine serodiagnosis and treatment of date-palm pollinosis.

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References

1. EL-ASFAHANI AMA, YOUSEF A, ZAHRAN F. Serum IgG levels in normal Saudi adults and patients with clinical allergic rhinitis. King Abdulaziz Med J 1982: 2: 23.
2. BOIRON P, DROUHET E, DUPONT B. Enzyme-linked immunosorbent assay (ELISA) for IgG in bagasse workers' sera: comparison with counter-immunoelectrophoresis. Clin Allergy 1987: 17: 355–63.
3. BRUCE CA, BIAS WB, NORMAN PS. Studies of HLA antigen frequencies, IgE levels and specific allergic sensitivities in patients having ragweed hay fever, with or without asthma. Clin Exp Immunol 1976: 25: 67.
4. FORD SA, BALDO BA. Identification of Bermuda grass pollen allergens by electroblotting. J Allergy Clin Immunol 1987: 79: 711–20.
5. HARFI HA, KWAASI AAA, TIPIRNENI P, PARHAR RS, GRAFF-LONNEVIG V, AL-SEDAIRY ST. Characterisation of antigens and allergens of date palm (*Phoenix dactylifera*) pollen: immunological assessment of atopic patients using whole extract and its fractions. Allergy 1992: 47: 535–44.
6. KAUFMAN HS, KELLY PT, MODIN GW. Antigen recognition in Chinese and Caucasians. Ann Allergy 1984: 53: 135–7.
7. KROUTIL LA, BUSH RK. Detection of *Alternaria* allergens by Western blotting. J Allergy Clin Immunol 1987: 80: 170–6.
8. KWAASI AAA, TIPIRNENI P, HARFI H, PARHAR RS, GRAFF-LONNEVIG V, AL-SEDAIRY ST. Date palm (*Phoenix dactylifera*) pollen is a potent allergen. American College of Allergy and Immunology. 48th Annual Meeting, New York. Abstract No. 31. Ann Allergy 1992: 68: 78.
9. LAEMMLI UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970: 227: 680.
10. LANGONE JJ. Radioiodination by use of the Bolton-Hunter and related reagents. Methods Enzymol 1980: 70: 221–5.
11. LEOFFLER JA, CAWLEY LP, MOEDER M. Serum IgG levels: correlation with skin test sensitivity. Ann Allergy 1973: 31: 100.
12. PARSONS NJ, KWAASI AAA, TURNER JA, et al. Investigation of the determinants of the survival of *Neisseria gonorrhoeae* within human polymorphonuclear phagocytes. J Gen Microbiol 1981: 127: 103–12.
13. PARSONS NJ, KWAASI AAA, PATEL PV, NAIRN CA, SMITH H. A determinant of resistance of *Neisseria gonorrhoeae* to killing by human phagocytes: an outer membrane lipoprotein of about 20 kDa with a high content of glutamic acid. J Gen Microbiol 1986: 132: 3277–87.

14. Pfei T, Schwabl U, Ulmer WT, Konig W. Western blot analysis of water-soluble wheat flour (*Triticum vulgaris*) allergens. Int Arch Allergy Appl Immunol 1990: 91: 224–31.
15. Riott IM, Brostoff L, Male DK. Immunology. 2nd ed. Edinburgh: Churchill Livingstone, 1989: 193.
16. Schwartz BD, Shreffler DC. Genetic influences on the immune response. In: Parker CW, ed. Clinical immunology. Vol. I. Philadelphia: WB Saunders, 1980: 49–85.
17. al-Shalan AA, al-Fray A, Reilley H, al-Hussein KA, Wilson DJ. Inhalant allergens in patients with allergic rhinitis in Riyadh. Ann Saudi Med 1989: 9: 331–43.
18. Shen HD, Wang SR, Tang RB, Chang ZN, Su SN, Han SH. Identification of allergens and antigens of Bermuda grass pollen by immunoblot analysis. Clin Allergy 1988: 18: 401–9.
19. Shen HD, Choo KB, Wang SR, Lin WL, Chang ZN, Han SH. Immunoblot analysis of components of *Penicillium notatum* recognised by human IgE antibodies. J Allergy Clin Immunol 1991: 88: 802–7.
20. Sorensen H, Gravesen S, Lind P, Schwartz B, Ashoor AA, Maglad S. The occurrence of indoor allergens in Saudi Arabia. Ann Allergy 1985: 54: 530–3.
21. Sorensen H, Ashoor AA, Maglad S. Perennial rhinitis in Saudi Arabia: a prospective study. Ann Allergy 1986: 56: 76–80.
22. Su SN, Lau GX, Tsai JJ, Yang SY, Shen HD, Han SH. Isolation and partial characterization of Bermuda grass pollen allergen, BG-60a. Clin Exp Allergy 1991: 21: 449–55.
23. Voller A, Bidwell DE, Bartlett A. Enzyme immunoassays in diagnostic medicine. Bull World Health Organ 1976: 53: 55–65.

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