

Identification of potential allergens in white oak (*Quercus alba*) pollen by immunoblotting

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Aqueous extracts of white oak pollen were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. The nitrocellulose membranes were blocked with phosphate-buffered saline 15% nonfat dry milk, incubated with dilutions of sera from atopic or control subjects, and probed with a radiolabeled or peroxidase-labeled antihuman IgE. The IgE binding bands were detected by autoradiography or enzymatic reaction; 45 to 50 protein bands were observed in silver-stained gels. IgE from 30 of the 38 sera tested from oak-sensitive subjects bound to 23 bands with molecular weights (MWs) between 106 to 108 kd (band 1) and 13.2 to 15.2 kd (band 23). No band was recognized by sera of every patient. Band 5 (MW 74.0 to 77.9 kd) and band 21 (MW 16.2 to 17.7 kd) were recognized by 71% of the patients' sera. Multiple bands were recognized by 30% to 50% of the sera tested. All patients who were skin test positive to oak by prick testing had positive immunoblots. Of 12 patients positive by intradermal skin testing, only four patients had positive immunoblots. The average number of allergens recognized by a single patient was 6.6. The maximum number of allergens to which any individual reacted was 18; the minimum number was one. Extracts separated under nonreducing conditions resulted in aggregates that did not enter the polyacrylamide gel. Of the protein that did enter the gel, the higher MW species elicited banding patterns similar to patterns observed under reducing conditions, whereas lower MW IgE binding bands were lost. These data suggest that the extractable proteins of white oak pollen contain multiple proteins that are potentially allergenic. (J ALLERGY CLIN IMMUNOL 1989;84:9-18.)

The genus *Quercus* (oaks) represents one of the largest and most diverse genera of trees; 371 species of oak have been described in the western hemisphere,¹ of which most are found in Central America. Trelease¹ described 84 species within the confines of the United States, whereas other investigators suggest slightly fewer species.² Of the U.S. oaks, 43 species are found predominantly in the East, and 36 to 41 species are largely located west of the Mississippi River. In addition to being a large genera, the oaks have diverse characteristics, including both large woody varieties and small scrub types. The range of

Abbreviations used

SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
NC:	Nitrocellulose
RAHIgE:	Rabbit antihuman IgE
NFDM:	Nonfat dry milk
DDW:	Distilled/deionized water
PBS:	Phosphate-buffered saline
kd:	Kilodalton
2-ME:	2-Mercaptoethanol
GAHIgE:	Alkaline phosphatase goat antihuman IgE
NTB:	Nitro blue tetrazolium
BCIP:	5-Bromo-4-chloro-3-indolyl phosphate
MW:	Molecular weight

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the oak genus also is diverse. Several species, such as white oak (*Q. alba*) and northern red oak (*Q. rubra*) are found over a wide range, whereas other species, such as the Georgia oak (*Q. georgiana*) or the Mex-

ican dwarf oak (*Q. depressipes*) grow in such restricted areas that they are largely overlooked in most texts.² In addition to the recognized species, the oaks cross hybridize at high frequency, and many of the hybrids are fertile. This greatly increases the number of oak trees, particularly in areas in which several species of oak coexist.³

Because of the large number of species of oak, the large number of individuals within the area of some of the more prominent species, the large amount of pollen that are shed by the oaks, and, as demonstrated in this article, the high allergenicity of the extractable pollen proteins, the oaks represent one of the most frequent causes of seasonal pollinosis among the trees. Indeed, in the Midwest, oak pollen is the most frequent cause of tree-seasonal pollinosis.⁴

Despite the large numbers of patients that are allergic to oak pollen, little is known of the allergens in oak pollen. Several groups of investigators, including our own investigative group, have examined oak-pollen allergens and have described a number of IgE binding bands by immunoblotting.^{5,6} Recently, however, we have noted that many proteins in oak-pollen extracts nonspecifically bind immunoglobulins when nonionic detergents are included in the incubation media.⁷ Thus, a reevaluation of immunoblotting to oak-pollen proteins with alternative technology appears warranted.

In the present study, we examined the IgE binding proteins in white oak pollen by immunoblotting with techniques that avoid the use of nonionic detergents and yet yield high "signal to noise ratios."⁷ These data indicate that oak pollen from a single oak species contains a broad range of proteins that are capable of binding IgE in oak-allergic patients.

MATERIAL AND METHODS

Material

White oak pollen was obtained from International Biologicals Corp., Piedmont, Okla. The pollen was ascertained to be pure by light microscopy of both stained and unstained specimens. Alternatively, the mature inflorescences from a single well-characterized white oak tree were collected and brought to our laboratory. The released pollen was allowed to fall onto polished brown paper, and the pollen was then purified by sequential sieving. This pollen was also ascertained to be pure by light microscopy. Extracts of these two pollen sources elicited identical results on Western blotting and are used interchangeably. ¹²⁵I-labeled RAHIgE was obtained from Pharmacia Diagnostics, Piscataway, N.J. This preparation had a specific activity of approximately 250 kBq (6.8 μ Ci)/ μ g of protein. Affinity-purified GAHIgE and BCIP/NBT substrate kit were obtained from Kirkegaard & Perry Laboratories, Gaithersburg, Md. NFD, Food Club brand, was obtained from Topco Associates, Skokie, Ill.

Material for SDS-PAGE and for immunoblotting were obtained from Bio-Rad Corp., Palo Alto, Calif., as previously described.⁸ The colloidal gold reagent for the staining of protein blots (AuroDye-Forte) was obtained from Janssen Pharmaceutical, Piscataway, N.J. DDW was produced with the Milli-Q system (Millipore Corp., Bedford, Mass.). All other chemicals were reagent grade or better and were obtained from VWR Scientific, Chicago, Ill.

Sera

Sera were obtained from 47 patients with positive reactivity to oak mixtures determined by prick and/or intradermal skin testing. Of these patients, five had been previously diagnosed as oak sensitive and were receiving immunotherapy, whereas the remaining patients were newly diagnosed. Nine of the sera were prepared from blood that had been collected 16 to 48 hours before being spun down. None of these sera demonstrated IgE binding to oak-pollen proteins separated by SDS-PAGE, and these sera are not considered in the data analysis. The remaining sera were processed within 120 minutes of collection.

Atopic nonoak-sensitive control subjects were defined as individuals with skin reactivity to a minimum of four aeroallergens but negative reactivity to oak based on both prick and intradermal skin testing. Nonatopic control sera was obtained from individuals with no history of allergic disease and no skin reactivity to a panel of 48 seasonal and perennial allergens.

Methods

White oak pollen was extracted in DDW at a ration of 1 gm of pollen to 10 ml of water (1:10 wt/vol) for 48 hours at 4° C. The extract was clarified by centrifugation at 170 g for 15 minutes at 4° C in a Beckman J-6 centrifuge (Beckman Instruments, Inc., Irvine, Calif.), followed by centrifugation at 16000 g for 30 minutes at 4° C in a Beckman J-21M centrifuge. The clarified extract was filtered through 0.45 and 0.22 μ m Millipore filters (Millipore Corp.). Each extract was then concentrated twofold in a ProDiCon (Bio-Molecular Dynamics, Beaverton, Ore.) vacuum filtration apparatus, refiltered as above, mixed with an equal volume of sterile glycerin, and stored at 4° C.

SDS-PAGE, under reducing conditions, was carried out on single-well 0.75 mm thick 15% "minigels" according to the buffer system described by Laemmli,⁹ and was previously described according to Wedner and Bass.¹⁰ In some cases the 2-ME was omitted from the Laemmli "disintegration buffer,"⁹ and the extract was run under nonreducing (nondissociating) conditions on 15% or 10% acrylamide gels. PAGE was carried out at 22 mAmp per gel until the dye front had reached the bottom of the gel. The gels were immediately equilibrated briefly in 0.025 mol/L of Tris HCl (pH 8.3), 0.192 mol/L of glycine, 20% methanol (transfer buffer), and blotted onto 0.22 μ m NC paper with a Bio-Rad transblot system.¹¹ Transfer was carried out for 120 minutes at 90 V. Adequate transfer of protein to the NC sheets was demonstrated by fixing the SDS gels in sulfosalicylic acid/trichloroacetic acid according to the method described by Guevara¹² and by staining the residual protein

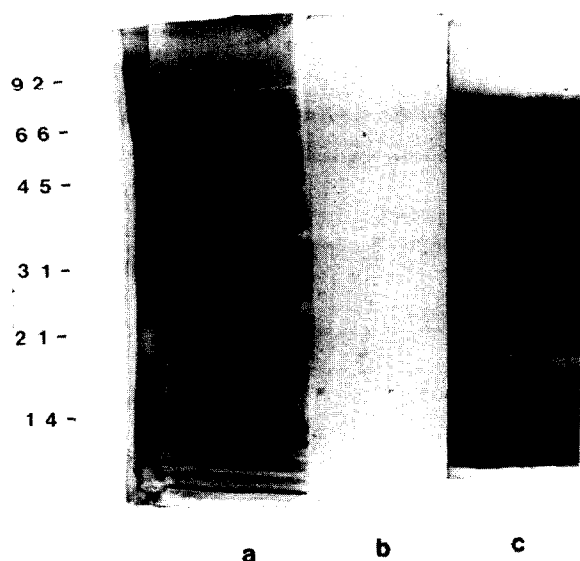


FIG. 1. Staining of SDS gels of white oak-pollen extract and the corresponding NC blot. *Lane a* demonstrates the banding pattern of white oak-pollen extract after SDS-PAGE and staining of the gel with silver. In *lane b*, only residual amounts of white oak pollen remain in the gel after staining with the same silver stain. *Lane c* demonstrates the colloidal gold-staining pattern of white oak-pollen extract bound to NC. The NC staining pattern is compressed compared to *lane a* because of shrinkage of the NC sheet during staining. The lane to the left of *lane a* corresponds to the molecular weight markers. The weights are expressed in kilodaltons.

with the Rapid Ag-Stain kit (ICN Pharmaceuticals, Irvine, Calif.), as described in the product literature. In addition, a strip was cut from the NC sheets and stained with colloidal gold, allowing visualization of a 5 to 10 mm full-length section of blotted pollen proteins.

The remainder of the NC sheets were air-dried and used immediately or stored at -20°C . For immunoblotting, the standard blotting solution contained 20 mmol/L of phosphate (pH 7.35), 154 mmol/L of NaCl, and 5% NFDm (blocking buffer). This solution was used to block all the unbound sites on the NC membranes, all washing steps, and for the dilution of all sera, as described below. To block unbound sites, the NC sheets were incubated in blocking buffer for 90 minutes at room temperature with constant agitation and with one change of the blocking buffer. The sheets were then cut into 4 mm strips; each strip was transferred to an individual well in an Accutran disposable incubation tray (Schleicher & Schuell, Inc., Keene, N.H.); all further steps were carried out in this tray.

Sera from atopic oak-sensitive, atopic nonoak-sensitive, and nonatopic subjects were diluted 1:2 with blocking buffer, and 0.5 ml of the diluted sera was added to each individual well. The plates were sealed to prevent evaporation, and the NC strips were incubated for 8 hours at room temperature with constant agitation for immunoblots performed with ^{125}I -labeled RAHIgE and for 16 hours at 4°C

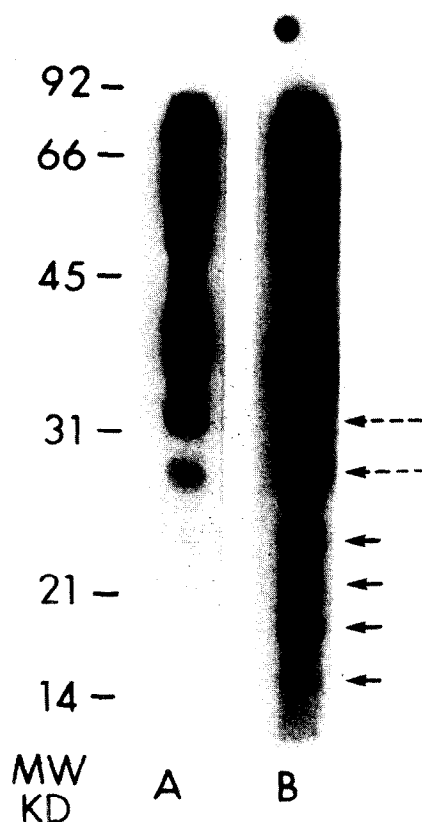


FIG. 2. Effect of autoradiographic exposure time on the detection of IgE binding bands. A single patient sera are illustrated. Of the multiple autoradiographic exposures, the 5-day, *lane A*, and 12-day, *lane B*, are illustrated. The solid arrows indicate four IgE binding bands not observed in the 5-day exposure but detected in the 12-day exposure. The broken arrows indicate two bands well demarcated on the 5-day exposure but blurred on the 12-day autoradiograph because of overexposure.

for immunoblots performed with GAHIgE. The sera were removed, and the strips were washed twice with 1.0 ml of blocking buffer for 20 minutes. One milliliter of ^{125}I -labeled RAHIgE diluted in blocking buffer to contain 50,000 CPM or 1 ml of GAHIgE diluted 1:500 in blocking buffer was added to each well. For isotopic detection, the wells were incubated overnight with constant agitation. The wells were washed twice for 20 minutes in blocking buffer, as described above, once in DDW, and air-dried. The strips were then placed against Kodak X-omat (Eastman Kodak Co., Rochester, N.Y.) film in cassettes containing a Cronex (Eastman Kodak Co.) intensifying screen, and the cassettes were stored at -70°C . Autoradiography was for varying times (5 to 21 days) on each blot to assure that weakly binding bands were not missed. In each case, control strips were exposed for 21 days to assure that they had indeed bound no ^{125}I -labeled RAHIgE. For enzymatic detection of bound IgE, the strips were incubated for 120 minutes at room temperature and washed twice in blocking

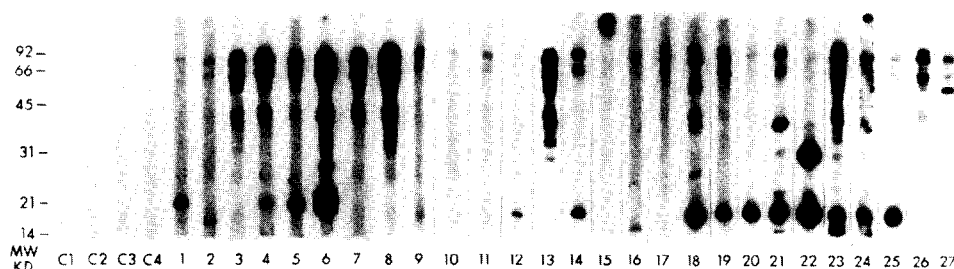


FIG. 3. White oak-pollen immunoblots (set 1). The controls, *lanes C1-C4* are C1, phosphate-buffered saline; C2, sera from nonatopic subjects; C3, sera from atopic nonoak-sensitive subjects; C4, sera from atopic nonoak-sensitive subjects. The control lanes were exposed for 21 days. *Lanes 1-27* represent the white oak immunoblotting patterns for each of 27 oak-allergic patients. Exposure of lanes 1-27 was for 12 days.

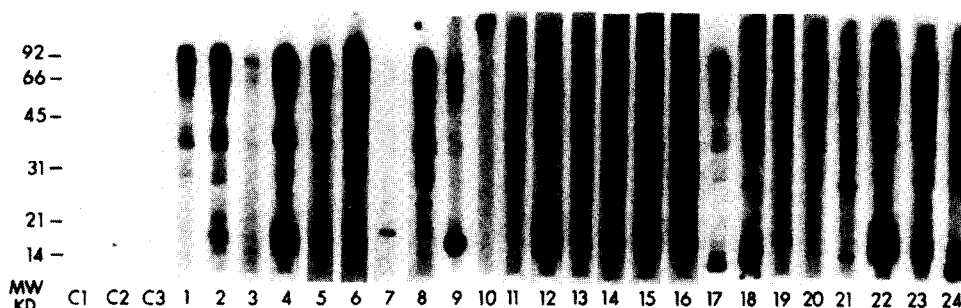


FIG. 4. White oak pollen immunoblots (set 2). the control lanes *C1-C3* are C1, sera from nonatopic subjects; C2, sera from atopic nonoak-sensitive subjects; C3, sera from atopic nonoak-sensitive subjects. *Lanes 1-24* represent the white oak immunoblotting patterns for 24 oak-allergic patients. Autoradiographic exposure was for 10 days for lanes 1-24 and 21 days for C1-C3.

buffer and once in phosphate-buffered saline. The strips were then incubated in BCIP/NBT in Tris buffer, pH 9.5, according to the manufacturer's instructions. The color reaction was allowed to develop until no further staining was observed, usually 2 to 3 hours.

RESULTS

The electrophoretic transfer of white oak-pollen extracts separated by SDS-PAGE was very efficient over the entire MW range. As illustrated in Fig. 1, there was little or no residual protein remaining in the SDS gel after transfer. The colloidal gold stain of the transferred protein clearly demonstrated that all the proteins found in the SDS gel had transferred to NC, indicating that significant pollen allergens would not be missed because of ineffective transfer from the SDS gel to the NC sheets. Minor differences in the appearance of the banding pattern between the silver-stained SDS-polyacrylamide gel and the immunoblot stained with colloidal gold most likely reflect the fact that different proteins bind more or less quantities of the stains used for protein visualization.

The intensity of the bands detected by autoradiography varied greatly from patient to patient. For this reason, these data analysis was carried out with autoradiographs exposed for various periods of time, and the actual data for the total number of bands observed in a single individual or the total number of individual sera that detected a given band represent an aggregate analysis. For the sake of brevity, only a single autoadiograph for two individual experiments is presented (see below). The effect of varying times of autoradiography is illustrated in Fig. 2. Autoradiography for 2 to 5 days produced nine IgE binding bands. Further autoradiography for 12 days revealed an additional four bands not observed with the shorter exposure. However, two bands had become obscured because of overexposure of the bands close to them.

The use of an alkaline phosphatase-labeled GAHlgE produced results that were comparable to results with ^{125}I -labeled RAHlgE. Some of the less reactive bands were not as distinct as bands detected with long radiographic exposures but were nonetheless observable.

Thirty-eight sera were then tested by immunoblotting, along with appropriate sera from nonatopic and atopic nonoak-sensitive control subjects. Thirty of the 38 sera tested elicited one or more positive bands detected with ^{125}I -labeled RAHIgE. The results of two experiments are illustrated in Figs. 3 and 4 and represent 10- and 12-day exposure, respectively. It is evident from these two figures that oak-sensitive patients generate IgE that is directed against a broad spectrum of pollen proteins. Of the 45 to 50 bands detected by silver staining of the SDS-PAGE, 23 bands bound to IgE in one or more patient's sera (Table I). The MW range of the potential oak-pollen allergens was from 106 to 108 kd (band 1) to 13.2 to 15.2 kd (band 23) (Table I).

The distribution of IgE binding bands (Table I) was compiled from two sets of experiments. Experiment 1 contained 27 positive sera, whereas experiment 2 contained 24 positive sera. Six patients in set 1 were not contained in set 2, whereas three patients in set 2 were not contained in set 1. The frequency distribution for the 23 allergens was remarkably similar for both sets. Statistical analysis of the two data sets revealed marked concordance by both parametric and non-parametric analysis. An analysis of variance (one-way) for the entire data sets revealed that the two sets are not significantly different ($F = 1.44$, $V = 1.88$, $T = 1.20$, and $p = 0.24$). When the patients common to both sets were analyzed, the mean difference between the two sets was 0 ± 0.6 .

No single IgE binding band was recognized by all 30 positive sera (Table I). The most frequent were bands 5 (74.0 to 77.9 kd) and 21 (16.2 to 17.7 kd) that bound IgE in sera of 71% of the patients tested. Eight other bands were positive in 25% to 54% of the sera from oak-sensitive subjects. Two broad groups of IgE binding bands were discernible. These are the groups including bands 7 through 11 (group 7/11) and bands 14 through 17 (group 14/17). No single serum was reactive to all of the bands in group 7/11. However, two sera were reactive with four of the five bands, and seven sera reacted to three of the five species. Only four individuals were reactive with a single member of this group of allergens. Six sera were reactive with all four members of group 14/17, whereas four sera bound two members of this group and four sera bound to only a single band. The remainder of the bands were detected with less frequency. Bands Nos. 1, 4, 6, 12, 13, 18, and 19 were detected by fewer than four sera.

The number of allergens detected by a single atopic serum ranged from one to 18 (Fig. 5). The mean number of bands was 6.6, and the median number was 5. Twelve sera reacted with less than four bands,

TABLE I

Band No.	MW range* (kd)	No. of sera† (N = 27)
1	106-108	1
2	83.2-84.9	5
3	79.3-81.9	9
4	78.2	2
5	74.0-77.9	17
6	73.7	1
7	69.7-72.8	13
8	66.7-69.1	14
9	63.4-66.3	12
10	60.2-62.7	13
11	56.2-58.2	10
12	51.1-56.1	2
13	47.3-48.9	3
14	39.1-45.6	13
15	36.5-39.2	12
16	34.5-35.9	6
17	29.5-31.3	14
18	24.1-25.3	14
19	22.1-23.8	4
20	19.3-20.9	8
21	16.2-17.7	17
22	15.2-16.0	9
23	13.2-15.0	9

* The molecular weight ranges (as determined by multiple immunoblots) are listed for each of 23 bands.

† Number of sera with IgE binding to each individual band (N = 27)

and 10 individuals were reactive with 10 allergens or more. Five sera reacted with 10 bands, two sera reacted with 16 bands, and one individual each was reactive with 12, 13, and 18 bands. There did not appear to be a relationship between the degree of sensitivity, as determined by symptoms or skin test reactivity, and the number of positive IgE binding bands that were detected by a single serum, with the exception that all eight sera, appropriately collected and not demonstrating reactivity against any of the oak-pollen bands, were obtained from patients that were positive by intradermal and not by prick skin testing.

When the patterns of reactivity among the various sera tested were examined, the spectrum of reactivity to SDS-PAGE-separated oak-pollen proteins was extremely diverse. There were some relatively similar patterns but of the 30 positive individuals tested, no two reacted with the same spectrum of IgE binding bands.

To examine further the distribution of IgE-containing bands, oak-pollen extracts, separated under reducing and nonreducing conditions, were compared

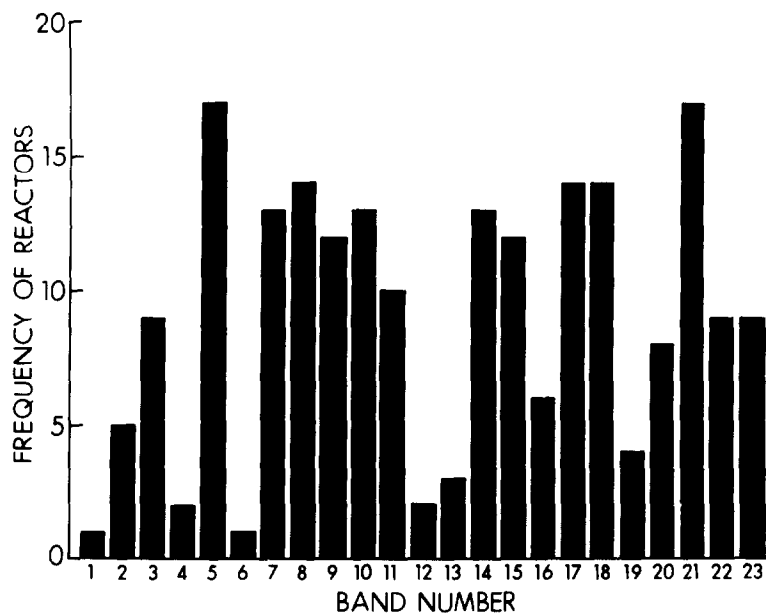


FIG. 5. Distribution of oak reactivity in 30 oak-sensitive patients. The vertical bars represent the number of positive bands for each of 30 patients.

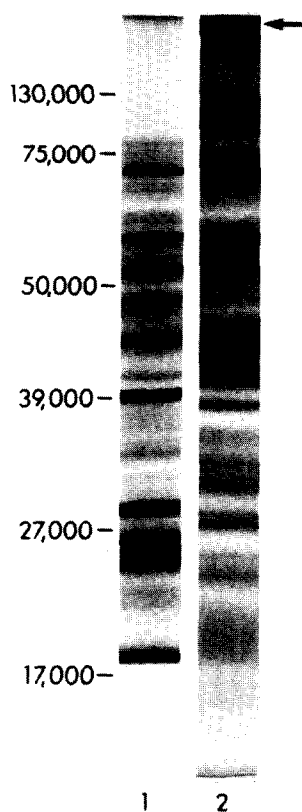


FIG. 6. Silver-stained SDS-PAGE of white oak-pollen extracts run under reducing and nonreducing conditions. White oak extract was incubated with or without 2-ME; the extract was separated on 15% polyacrylamide gel and stained by silver impregnation. Lane 1, reducing conditions; lane 2, nonreducing conditions. The arrow indicates the presence of aggregated protein that was unable to enter the 15% polyacrylamide gel.

with the GAHIgE enzymatic detection system. As demonstrated by silver staining (Fig. 6), failure to include 2-ME in the "disintegration buffer" led to extensive aggregation of extract protein, as evidenced by the inability of much of the protein to penetrate 15% polyacrylamide gels. Similar results were observed with 10% polyacrylamide (not presented). Much of the unreduced proteins that did enter the gels migrated at MWs in excess of 30,000 daltons. In addition, the silver-stained bands were less distinct in unreduced compared to reduced gels. There were a number of bands that appeared to migrate identically under reduced and nonreduced conditions.

Direct comparison of IgE binding bands from reduced and nonreduced gels transferred to NC revealed less distinct bands observed in the nonreduced transfers. Nonetheless, when IgE binding bands were examined with a pool of sera from oak-sensitive subjects, most of the bands were identical in both electrophoresis systems. Specifically, band 5 and group 7/11 were not altered when white oak-pollen extract was tested under nonreducing conditions. In contrast, a number of lower MW bands were not observed when reduced and unreduced gels were compared (Fig. 7). Use of individual patient sera elicited similar results. As illustrated in Fig. 8, white oak extract run under nonreducing conditions elicited similar patterns for the higher MW range IgE binding bands and absent or greatly reduced binding in the lower MW range.

DISCUSSION

Oak pollen is a major cause of seasonal pollinosis in the United States.¹³ Because previous immunoblot

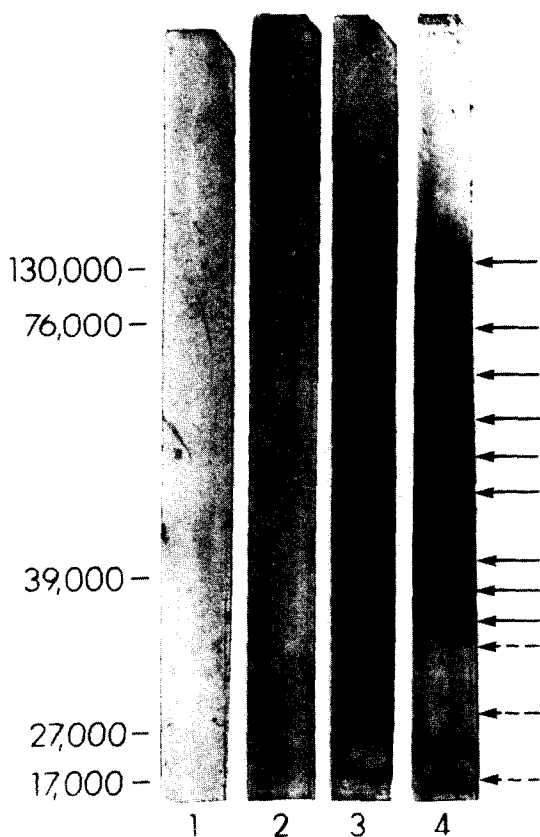


FIG. 7. Immunoblot of white oak extract separated under reducing and nonreducing conditions. Immunoblots separated in the presence or absence of 2-ME were probed with a pool of sera from oak-sensitive subjects, and bound IgE was detected with GAHlgE. Lane 1, nonreduced conditions, control sera; lane 2, reduced conditions, control sera; lane 3, nonreduced conditions, sera pool from oak-sensitive subjects; lane 4, reducing conditions, sera pool from oak-sensitive subjects. The *solid arrows* indicate bands that are similar under reduced and nonreduced conditions; the *broken arrows* indicate bands detected under reducing conditions but absent when nonreducing conditions were used.

analysis of oak-pollen allergens^{5,6} may be compromised by nonspecific binding of labeled anti-IgE to the NC-bound pollen proteins caused by nonionic detergents,⁷ in the present study we avoided the use of nonionic detergents and used NFDM as the major blocking agent. The effectiveness of NFDM is demonstrated by the lack of reactivity when sera from nonatopic or atopic nonoak-sensitive subjects are examined. Despite increasing the autoradiographic exposure to 21 days, no ¹²⁵I-labeled RAHlgE bound to the blotted oak-pollen proteins when sera that would not be expected to have IgE antioak were tested. No IgE was detectable with GAHlgE, despite leaving the BCIP/NBT reagent in contact with the strips for 16 hours with two changes.

Immunoblotting is capable of detecting binding of IgE to proteins separated by a variety of techniques.

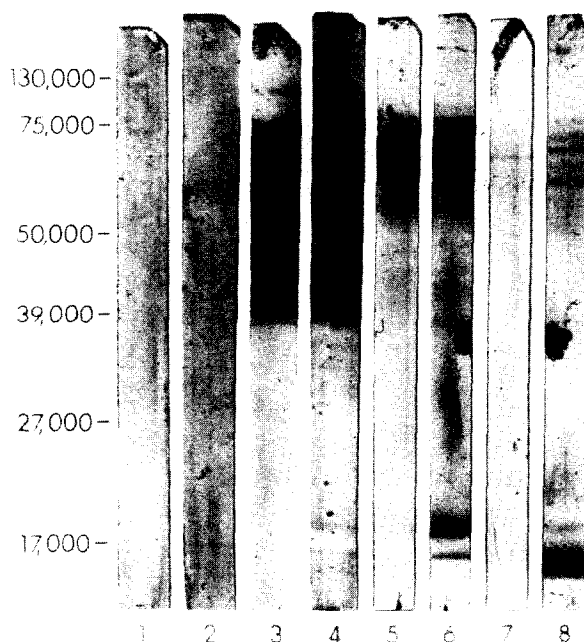


FIG. 8. Evaluation of individual patient reactivity to white oak extract separated under reducing and nonreducing conditions. Immunoblotting was carried out on white oak-pollen extracts separated with or without 2-ME. The binding of sera from individual oak-sensitive subjects was detected with GAHlgE. Odd numbered lanes were run under nonreducing conditions and even numbered lanes were run under reducing conditions. Lanes 1 and 2, control sera; lanes 3 and 4, patient M. B.; lanes 5 and 6, patient T. L.; lanes 7 and 8, patient V. B.

in this study with SDS-PAGE under reduced and non-reduced conditions. With this capability, it is an extremely efficient technique for comparison of the reactivity of individual patient sera to a single allergenic extract or of a pool of sera to different extracts of pollen from a single species or a variety of species. However, actual interpretation of the allergens in the IgE binding bands is not possible. The conditions used for the separation of proteins may lead to alterations in IgE binding epitopes, reducing the actual number of reactive sites. In contrast, identical epitopes may be present on several polypeptides that migrate differentially. This analysis may be further compromised in gels observed under reducing conditions, since multichain proteins with IgE binding epitopes on more than one chain will elicit multiple IgE binding bands for a single allergenic protein. Thus, the actual nature of the pollen proteins that bind IgE and are true allergens cannot be deduced by immunoblotting.

As performed in this study, the immunoblotting procedure involves the separation of proteins in the white oak extract under denaturing conditions. This technique has been criticized because denaturing the proteins might falsely increase or decrease the number of bands that react with specific IgE. In point of fact,

a number of workers have demonstrated that significant renaturation of the proteins occurs during the transfer of the protein to the NC sheets.^{14, 15} The renaturation is enhanced if SDS is excluded in the transfer buffers, as was performed in this study, and if the NC sheets are incubated in phosphate-containing buffers during the blocking step, which was also the technique used in this study.¹⁵ The renaturation of proteins bound to NC is further demonstrated by the fact that antibodies generated to denatured proteins may not react with these proteins when they are bound to NC unless specific steps are taken to prevent renaturation during the transfer process.¹⁶ The recent demonstration that proteins transferred to NC after SDS-PAGE are capable of stimulating reactive T cells is additional evidence for the renaturation of proteins bound to NC.¹⁷

The major problem with the detection of antigenic proteins appears to be the presence of residual SDS bound to protein that prevents both renaturation and the binding of specific immunoglobulins to the bound proteins. This problem can be minimized by the techniques described above and by the inclusion of low concentrations of nonionic detergents, such as NP-40¹⁸ or zwitterionic detergents,¹⁹ in the incubation media. As noted above, the inclusion of nonionic detergents in the incubation media in our study led to non-specific binding of immunoglobulins to the separated oak proteins, preventing us from using this technique.

The effect of conformational change on the reaction of specific IgE with allergens has been examined by Hoffman.^{20, 21} He demonstrated that IgE from individuals sensitive to Hymenoptera stings bound significantly better to proteins separated and transferred under nondenaturing conditions than to proteins separated and transferred under denaturing conditions. Thus, although we detected a large number of bands that bound IgE from oak-sensitive individuals, this must be considered an underestimate of the actual number of IgE binding proteins in oak-pollen extracts.

We were able to detect 23 bands on SDS-PAGE, run under reducing conditions, that bound IgE from one or more of the atopic sera tested. The MW range of the allergens detected was from >100,000 daltons to 13,200 daltons. No single band was detected by all positive patients. Two bands, Nos. 5 and 21, were clearly the most prominent; however, 10 other bands were detected by sera from 25% to 54% of the patients tested. In addition, there was a tendency for two groups of bands to be detected together. It should be pointed out that there were patients who reacted to only one member of these groups. This suggests that there may be both common and distinct epitopes among the proteins found in these bands. It is also

conceivable that these bands represent breakdown products that retain or lose allergenic epitopes or that they represent variable glycosylation of an identical core peptide.

Previous studies have demonstrated IgE binding proteins or bands in pollen from a variety of species of trees, grasses, and weeds. These studies used immunoblotting of both denatured and undenatured SDS-PAGE, crossed radioimmuno-electrophoresis, and physicochemical techniques.²²⁻³³ Because different extraction procedures and a number of techniques for detecting "allergens" have been used, direct comparison of IgE binding bands in oak pollen with other pollen extracts is not possible. However, the spectrum of allergenic bands in white oak pollen detected by immunoblotting appears to differ from the pattern described for other pollen extracts assayed by immunoblotting under similar conditions. The overall number of IgE binding bands is higher than described for other extracts, and the number of IgE binding bands as a fraction of the total number of bands is higher than that observed in most other studies of pollen allergens with immunoblotting. In addition, one of the most prevalent bands in our patient population is a high MW protein(s). Band No. 5 has an MW of 74.0 to 77.9 kd and is detected by 71% of the individuals tested.

Of 12 patients who were skin test positive to oak by intradermal but not prick skin testing, only four patients had positive immunoblots. Since intradermal skin testing has been reported to result in nonspecific tests in some cases,³⁴ it is possible that some of the positive intradermal skin tests were caused by non-specific skin test reactivity. Immunoblotting may thus provide a sensitive means of distinguishing between those intradermal reactions mediated by antigen-specific IgE and reactions caused by nonspecific skin test reactivity.

To gain further insight concerning the nature of the IgE binding bands from white oak pollen, immunoblotting was performed on extracts observed under both reducing and nonreducing conditions. The analysis of oak-pollen extracts under nonreducing conditions was compromised by the extensive aggregation that occurred when 2-ME was omitted. Similar results were reported for *Parietaria judaica* by Ford et al.³⁵ For this reason it is not possible to state that IgE binding bands in the lower MW range not detected with nonreducing conditions were lost by being bound up in large aggregates or because they represented a single polypeptide chain of a larger allergenic protein. The appearance of bands under reducing conditions in ryeglass has led to speculation that dissociation may occur in vivo.³⁶ The difficulties of nonreducing con-

ditions notwithstanding, one of the two bands that bind IgE from the largest number of individuals, No. 5, was largely unchanged, as were the group 7/11. This strongly suggests that one or more of the allergenic proteins in these bands exist normally as single chain polypeptides. In this respect, the comparison of reduced and nonreduced extracts has proved useful. However, as pointed out by Tovey et al.,³⁷ for routine use, reduced (dissociated) conditions most likely represent the procedure of choice.

The present study of white oak-pollen extracts, like all studies of allergenic extracts with Western blotting technology, reveals only those SDS-PAGE bands that contain proteins or polypeptides that bind IgE from patients known to be oak sensitive. Clearly it does not reveal which of these bands contain the proteins that are responsible for the symptoms of allergic diseases. Nonetheless, it is not unreasonable to assume that all or a fraction of the bands detected contain true allergens. Thus, Western blotting provides an ideal starting point for the purification and characterization of oak allergens. It also may provide a simple, reproducible method for the comparison and standardization of oak-pollen extracts.

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