

Comparison by electroblotting of IgE-binding components in extracts of house dust mite bodies and spent mite culture

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Eight Dermatophagoides pteronyssinus extracts (three culture extracts, four mite body extracts, and the World Health Organization International Standard [IS]) were investigated by side-by-side sodium dodecyl sulfate-polyacrylamide gel electrophoresis, by electrotransfer to nitrocellulose, and by probing with a pooled serum from mite-allergic subjects. Representative body and mite culture extracts were compared by probing with individual sera, and both types of extract were also compared by RAST-inhibition studies. Extracts from the same source differed in the molecular weight (MW) of some of their IgE-binding components. In general, most IgE-binding components in culture extracts and the IS were in the 14 to 35 kd MW region, whereas extracts from mite bodies and one culture extract contained more IgE-binding components of higher MW (35 to 110 kd). Comparison of representative mite body and culture extracts by use of 22 separate sera resolved 26 and 19 IgE-binding components in the two extracts, respectively. Patterns of RAST inhibition produced by both types of extracts when they were used either as the inhibitor or as the allergosorbent demonstrated qualitative differences between the two types of extracts. These results demonstrate that mite extracts may differ considerably in their allergenic composition and emphasize the need for standardization of mite allergenic extracts and the reexamination of the suitability of the D. pteronyssinus IS.
(J ALLERGY CLIN IMMUNOL 1987;79:93-102.)

A prime requirement for an adequately standardized allergen extract used for diagnosis is that the extract will contain all the allergens produced by the natural source. For the house dust mite *D. pteronyssinus*, a widespread and major cause of allergic asthma,¹⁻³ this is not an easy task because the origin, number, and location of all the clinically important allergens have not been firmly established. In earlier attempts to identify important mite allergens, extracts were prepared from spent mite culture material, including mite feces,^{4,5} whereas in other such investigations mite bodies free of culture material were used as the source.⁶⁻⁸ Both types of extracts have been demonstrated to be potent in both in vitro and in vivo assays for total allergenicity, but none of these studies compared the complement of allergens in the two different types of extract.

Abbreviations used

CBB:	Coomassie brilliant blue
CRIE:	Crossed radioimmuno-electrophoresis
Der.p.I:	International Union of Immunological Societies classification, previously mite allergen P ₁ , Ag 42, and Dpt 12
Der.p.II:	International Union of Immunological Societies classification, previously mite allergen DpX
IS:	International standard
Kd:	Kilodaltons
MW:	Molecular weight
NC:	Nitrocellulose
PAGE:	Polyacrylamide gel electrophoresis
PBS:	Phosphate-buffered saline
SDS-PAGE:	Sodium dodecyl sulfate-PAGE
WHO:	World Health Organization

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Supported by the National Health and Medical Research Council of Australia.

Received for publication Dec. 20, 1985.

Accepted for publication July 5, 1986.

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The identification of the individual allergens in different mite extracts has previously been performed with CRIE. Lind⁹ and Lind et al.^{10, 11} have demonstrated the same five allergens in different proportions in extracts of mite bodies and in extracts from whole mite culture. The total numbers of mite allergens identified by CRIE have been reported as four,⁹ 11,⁸ and 18¹¹ in extracts of mite bodies, and five¹² and nine,¹⁰

in extracts of mite culture; however, five of the 18 allergens recognized in mite culture were also bound by the controls.¹¹ The identification of allergens by CRIE is dependent on several variables, including the hyperimmune rabbit antiserum used for precipitating the antigens, and since this varies both between laboratories and during a period of time from the same animal, it is difficult to compare the published results of the allergens recognized.

The manufacturers of commercial mite extracts regard details of their production as confidential, and the source of the material and the extraction procedures used are neither supplied nor available. However, it is generally believed that some extracts are derived from mite bodies and that other extracts are prepared from mite culture, possibly enriched for feces.

The significance of investigations into the source and preparation of mite allergens is illustrated by our experience with the WHO IS. The choice of this extract was made partly on the basis of the nonallergenic nature of the culture material used but, more significantly, because the most important allergens were considered to be present. However, only three allergens were detected in this extract in the initial study,¹³ and six allergens were detected in a later study.¹⁴ In our opinion, all that is currently available is a limited description, rather than a complete definition, of the full complement of mite allergens.

With an alternative technique, protein blotting¹⁵ for allergen identification, we were able to recognize more than 20 IgE-binding components in a mite body extract.¹⁶ This technique involves the separation of extract components by SDS-PAGE followed by their electrotransfer to NC and probing with sera from allergic patients and anti-IgE. With this technique resolution is determined by the MW of the antigens and is not dependent on an intermediary hyperimmune serum. With protein blotting and RAST inhibition, the qualitative compositions of extracts of mite bodies, spent mite culture, and the WHO *D. pteronyssinus* IS were compared in an attempt to establish which extracts are the most suitable for clinical application.

MATERIAL AND METHODS

Mite extracts

Mite culture extract C1 was prepared from 1 gm of a laboratory culture of mites grown on beef liver extract (Oxoid, London, England) passed through a 50 μ m sieve, extracted for 10 minutes in 10 ml of saline, centrifuged (18,000 $\times$ g for 20 minutes), and dialyzed against PBS overnight. The extract (1.73 mg of protein per milliliter) was stored at -20° C for 3 years and then concentrated about 15 times in Centricon 10 ultrafiltration tubes, MW

cutoff 10 kd (Amicon, Lexington, Mass.) before electrophoresis. Mite culture extract C2 was prepared from the above unsieved whole culture material extracted for 2 hours at 4° C, dialyzed, and then freeze-dried. Extract C3 was prepared by overnight extraction of mite culture material and was provided and described by Dr. M. Chapman.⁴ Except for approximately 1 week at ambient temperatures during air freight (preserved with 0.1% (w/v) sodium azide), the extract was stored for 3 years at between -70° C and -20° C. Extract W was the WHO IS reference preparation trial fill of vials 82/568 and can be considered identical to the main fill of vials 82/518.* The extract was supplied freeze-dried, and the protein content has been reported as 300 μ g per ampule by the Lowry method and 50 μ g per ampule by dye-binding assay.¹⁷ Extract M1 was prepared from 1 gm of clean dried mite bodies (Commonwealth Serum Laboratories, Parkville, Victoria, Australia; batch P549), homogenized on ice in 40 ml of PBS for 15 minutes with a blender coated with Teflon fluorocarbon resins, sonicated for 5 minutes, stirred at 0° C for 2 hours, centrifuged 30 minutes at 18,000 $\times$ g, concentrated by ultrafiltration to 8.7 mg/ml of protein, and stored at -20° C. Extract M2 was prepared by a similar procedure from mites from Commonwealth Serum Laboratories (batch 546), but extraction was for 1 hour. The extract was dialyzed (4° C) against PBS before storage for 3 years at -20° C and then was concentrated for electrophoresis by ultrafiltration to 20 mg/ml. Extract M3 was prepared by further extraction at 0° C for 22 hours of the centrifugation pellet from extract M1 and yielded 7½% of the total protein of the first extraction. Extract M4 was prepared from live mites cultured on liver powder and harvested on a sucrose gradient. Mites (25 mg wet weight) were homogenized by vortexing the wet mites with 1.5 mm glass beads for 5 minutes, followed by extraction in 0.25 ml of Tris buffer (pH 6.8) containing 4% SDS at room temperature and centrifugation at 10,000 $\times$ g for 15 minutes. The extract was prepared before electrophoresis and was not stored. Purified mite allergen P₁ was provided by Prof. T. A. E. Platts-Mills (Charlottesville, Va.). According to the proposed nomenclature of International Union of Immunological Societies, the antigen will be termed Der.p.I.¹⁴ All protein determinations were performed by the CBB-based protein assay (Pierce Chemical Co., Rockford, Ill.). The media that was used to culture mites for extracts C1, C2, and M4 had previously been extracted and heat denatured. Extracts of the media alone did not elicit a distinct protein-staining pattern on gels and did not bind IgE on immunoprobings. Extracts of the other media alone, used for extracts prepared outside the laboratory, were not available for study.

Sera

Human sera from mite-allergic subjects, human cord serum, and specific rabbit antisera against mite allergens Der.p.I and Der.p.II were all obtained as described previ-

*Ford A: Personal communication.

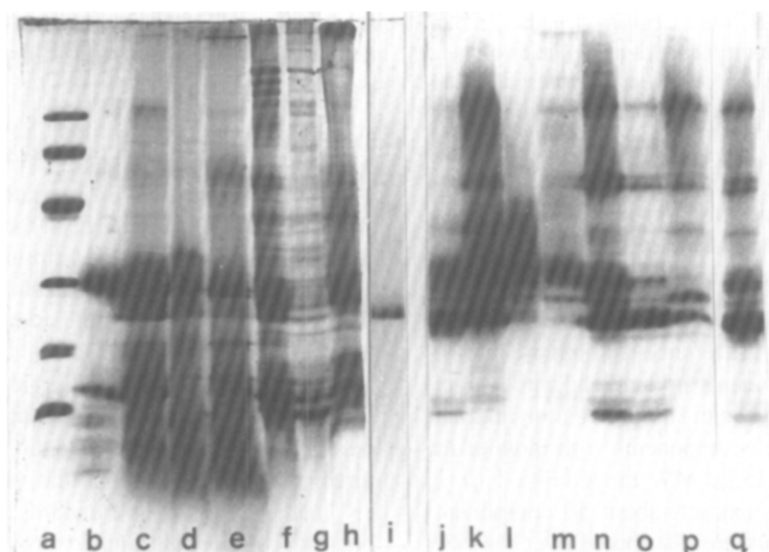


FIG. 1. MW markers and mite culture and body extracts separated by SDS-PAGE and stained for protein with CBB are illustrated in strips *a* through *h*. An autoradiograph of the immunoprobings of extracts for IgE-binding components (as described in Methods) and separated on the other half of the same gel is illustrated in strips *j* through *q*. Individual strips are as follows: *a*, MW markers 94, 67, 43, 30, 20, and 14.4 kd; *b* and *j*, feces-enriched mite culture extract C1; *c* and *k*, mite culture extract C2; *d* and *l*, International Union Immunological Societies IS extract W; *e* and *m*, mite culture extract C3; *f* and *n*, mite body extract M1; *g* and *o*, mite body extract M2; *h* and *p*, live mite body extract M4; *i*, purified mite allergen Der.p.I run on a separate gel to demonstrate relative position of this antigen; *q*, mite body extract M3. Protein loadings per well were as follows: C1, 28 μ g; C2, 116 μ g; C3, 82 μ g; M1, 142 μ g; M2, 57 μ g; M3, 125 μ g; M4, 137 μ g; W, 56 μ g; and Der.p.I, 5 μ g.

ously.¹⁸ One hundred sera from mite-allergic subjects were used to prepare the pooled serum. Radiolabeled anti-IgE was purchased from either Pharmacia (Uppsala, Sweden) or Kallestad Laboratories, Inc. (Austin Texas). Protein A was radiolabeled by a modification of the chloramine-T method.¹⁹

SDS-PAGE, electrotransfer, and immunoprobings

Gradient (9% to 27%) SDS-PAGE, electrotransfer, and immunoprobings with radiolabeled anti-IgE or protein A were performed as previously described.^{16, 18, 20} Briefly, SDS polyacrylamide gels (14 $\times$ 13 $\times$ 0.15 cm) were run overnight at 4° C, and the resolved components were electrotransferred to 0.1 μ m pore size NC. In all experiments the sample extraction buffer did not contain 2-mercaptoethanol. For the experiments described in Fig. 1, each well on the gel was loaded with 35 μ l of mite extract containing between 28 to 142 μ g of protein. For the experiments described in Figs. 2 and 3, a total volume of 300 μ l was loaded onto each half of the gel containing 1.11 mg and 1.26 mg of protein of mite body and mite culture extracts, respectively. After transfer to the NC, membranes were washed briefly with 10% v/v methanol/PBS and blocked by incubation with 0.1% v/v Tween-20 for 1 hour. For probing with individual sera, 4 mm wide NC strips were rocked overnight in tubes

at room temperature with 1.5 ml of 10% v/v sera from atopic patients in PBS/0.1% w/v sodium azide, followed by incubation with 40,000 cpm of radiolabeled anti-IgE for 8 to 12 hours. Sheets of NC containing transferred mite components were incubated with proportionately more sera from atopic patients and anti-IgE. When radiolabeled protein A was used to locate the binding of mite-specific rabbit antibodies (1:300), blocking was with 3% w/v of bovine serum albumin and 0.1% Tween to reduce background.²¹ Autoradiography was conducted with Kodak X-Omatic cassettes (Eastman Kodak Co., Rochester, N. Y.) and Fuji RX-safety film (Tokyo, Japan) for about 72 hours at -70° C. Autoradiographs were scanned on a Quick Scan densitometer (Helena Laboratories, Beaumont, Texas). Proteins in polyacrylamide gels were stained with CBB²² and on the NC with amido black. Densitometric scanning of the autoradiographs was performed with a Bio-Rad model (Bio-Rad Laboratories, Richmond, Calif.) 1650 scanning densitometer.

RAST-inhibition assays

Competitive RAST-inhibition assays were performed as previously described.²³ CNBr-activated paper discs were coupled with extract C1 or M2 (1.9 or 3.2 μ g per disc, respectively, established by direct RAST titration of each extract). Disc, inhibitor, and 12.5 μ l of pooled serum

from mite-allergic subjects were incubated together, in duplicate, with gentle agitation for 3½ hours. The disc-bound IgE was determined by overnight incubation with radio-labeled anti-IgE (10,000 cpm) and counted (10 minutes per disc).

RESULTS

Comparison of components in different mite extracts by protein staining and immunoprobings with IgE antibodies

Resolution by SDS-PAGE and CBB staining for protein revealed many differences in both the number and quantity of components in the eight mite extracts examined (Fig. 1, *b* through *i*). Spent culture extracts demonstrated about 20 components with most of the staining in the 10 to 35 kd MW range (Fig. 1, *b*, *c*, and *e*). In mite body extracts about 50 components were resolved, which were distributed over the MW range of 12 to 110 kd (Fig. 1, *f*, *g*, and *h*). The relative position of mite allergen Der.p.I run on another gel is illustrated in Fig. 1(*i*).

When the separated extracts illustrated in Fig. 1 (*b* through *h*) were transferred to NC and probed with a pooled serum from a mite-allergic subject, up to at least 20 IgE-binding components were identified (Fig. 1, *j* through *p*). Components of MWs 25 and 30 kd demonstrated the strongest binding of IgE, and these components were present in both types of extracts, although in extracts C3 and W the concentration of the 25 kd component was low, and in extract M4 the 30 kd component was virtually absent (Fig. 1, *l*, *m*, and *p*). Other components binding less IgE were resolved with apparent MWs of 14, 15, 16, 22, 27, 38, 52, 55, and 110 kd. The binding of IgE to the components did not closely correlate with the distribution or the extent of protein staining. Only a few of the many proteins in mite body extracts (Fig. 1, *f*, *g*, and *h*) bound IgE (Fig. 1, *n*, *o*, and *p*, respectively), and the 15 to 20 kd MW protein-staining components in extracts W (Fig. 1, *d*) and C3 (Fig. 1, *e*) bound little or no IgE (Fig. 1, *l* and *m*). When IgE was bound by these two extracts, it was usually diffuse binding despite the sharply stained protein bands on the corresponding gel. Conversely, protein bands on the corresponding gel. Conversely, proteins of MW 25 kd in extracts C1 and proteins of MWs 38, 52, and 95 kd in extract C2 (Fig. 1, *b* and *c*, respectively) demonstrated negligible protein staining of the gel but bound IgE when the NC transfer was probed (Fig. 1, *j* and *k*). Extract M3, prepared by a second prolonged extraction of mite bodies, contained a relatively high proportion of the 25 kd component (Fig. 1, *q*). This extract is not demonstrated stained

Immunoprobings of mite body and culture extracts with individual allergic human sera and rabbit antisera

When mite extracts M1 and C2 were run on separate halves of the same gels, transferred to NC, and probed with 22 separate sera from mite-sensitive subjects (Fig. 2, *A* and *B*, respectively), the cumulative pattern of IgE-binding from all the strips to these two extracts was similar to that with a serum pool (Fig. 1), but the resolution of IgE-binding components by individual sera was greatly improved.

The data for sera from allergic subjects illustrated in Fig. 2, *A* and *B*, is the composite of three gels (*strips* 7 to 14, 15 to 14', and 13' to 28) and includes three sera (9, 13, and 14) that were used twice (9', 13', and 14') to act as standards for comparing IgE-binding components and to provide a guide to the reproducibility of the system.

In the mite body extract, a total of 26 IgE-binding components were identified (Fig. 2, *A*, *strips* 7 to 28). Of these components, 15 bands demonstrated strong binding with at least one serum, and eight of these were recognized by nearly half of the sera. Their apparent MWs were ~95, ~53, 30, 27, 25, 18, 15, and 13 kd. The frequency and a semiquantitative assessment of IgE binding by the resolved and transferred mite body components is illustrated on the *left hand side* of strips 7 to 28 (Fig. 3, *A*) and in Fig. 3, *B*. Both the regions at 30 kd and 25 kd were resolved into at least two separate components (Fig. 2, *A*, *strips* 10 and 11), and the pair of components in Fig. 1 of MW ~52 and 55 kd were either bound as a pair (Fig. 2, *A*, *strip* 13) or as either band separately (Fig. 2, *A*, *strips* 14 and 16). Occasionally, IgE from a serum bound mainly to only a single minor component (Fig. 2, *A*, *strips* 17, 18, and 28). The region of binding associated with Der.p.II (15 kd) was also associated with at least two other components differing more or less in MW by only 2 to 3 kd that also bound IgE in many of these sera; indeed, binding to a single component in this region was only observed with the sera on strips 8, 12, 20, and 22. There was no binding of the anti-IgE to the strips after their incubation with IgE-free human cord sera (Fig. 2, *A*, *strip* 6).

The rabbit antisera against mite allergens Der.p.I and Der.p.II specifically identified these components in the mite body extract with MWs of 25 and 15 kd, respectively (Fig. 2, *A*, *strips* 3 and 4), with some slight additional binding to the two components of MWs 28 and 30 kd. These latter components were also slightly bound by the normal rabbit sera (Fig. 2, *A*, *strip* 5).

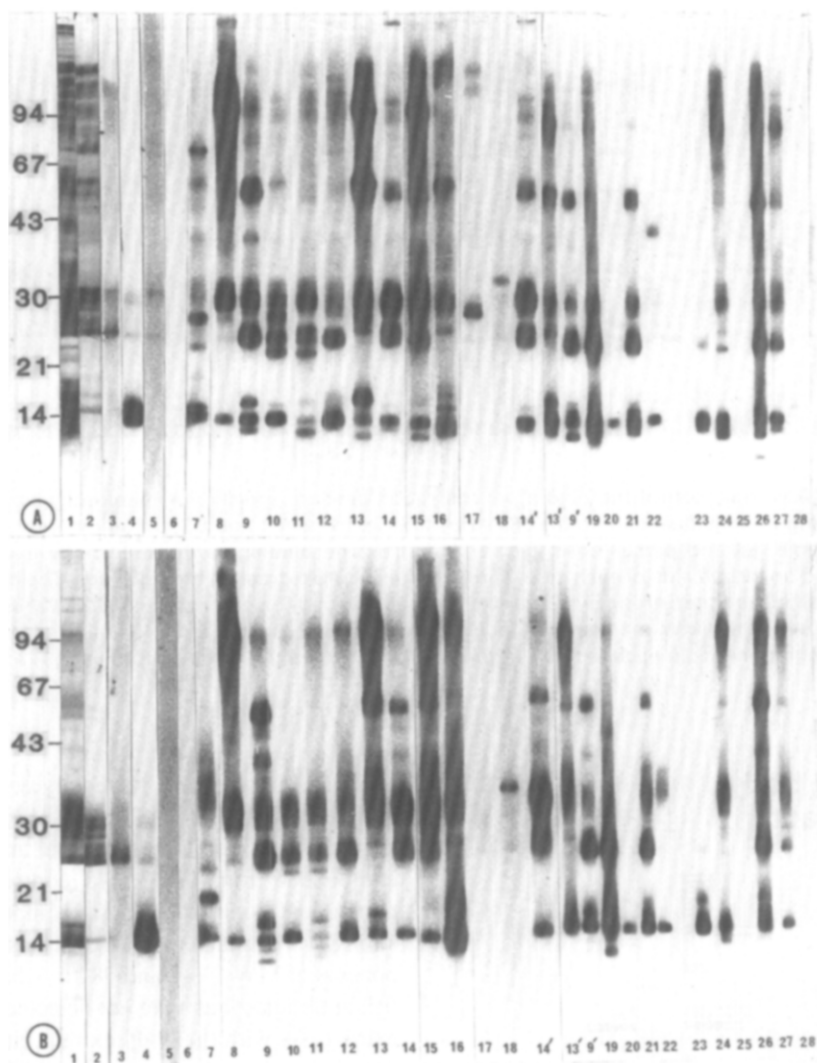


FIG. 2. A, Mite body extract M1 was separated by SDS-PAGE on three separate gels, and portions were either stained for protein or transferred to NC. The NC strips were either stained for protein or probed with rabbit antisera against specific mite antigens, control rabbit and human sera, and individual sera from mite-allergic subjects and autoradiographed (*strips 3 to 28*) to detect binding of antibodies as described in Methods. Strips are as follows: *1*, CBB staining of mite body components on gel; *2*, amido black staining of NC after transfer; *3*, rabbit antisera specific for mite antigen Der.p.I; *4*, rabbit antisera specific for mite antigen Der.p.II; *5*, normal rabbit serum control; *6*, IgE-free human cord serum control; *8 to 28*, sera from mite-allergic subjects. *Strips 8 to 14*, *15 to 14'*, and *13' to 28* were from separate gels. **B,** The same as for **A**, except that mite-culture extract C2 was separated on the other half of the gel.

When mite culture extract C2 was probed with the same 22 sera, a total of 19 IgE-binding components were resolved (Fig. 2, *B*). Of these components, 13 demonstrated strong binding with at least one serum, and four of these were bound by half or more of the sera. Their apparent MWs were 95, 32, 25, and 15 kd. These four components corresponded to five of the strong binding components in the mite body

extract since the two closely resolved components of MW, ~28 and 30 kd, in the mite body extract were resolved as a broad component of slightly higher MW in the culture extract. The frequencies and semiquantitative assessments of the binding of IgE by the different sera to the components in the mite body and mite culture extracts are illustrated on the *left and right hand sides* of Fig. 3, *B*.

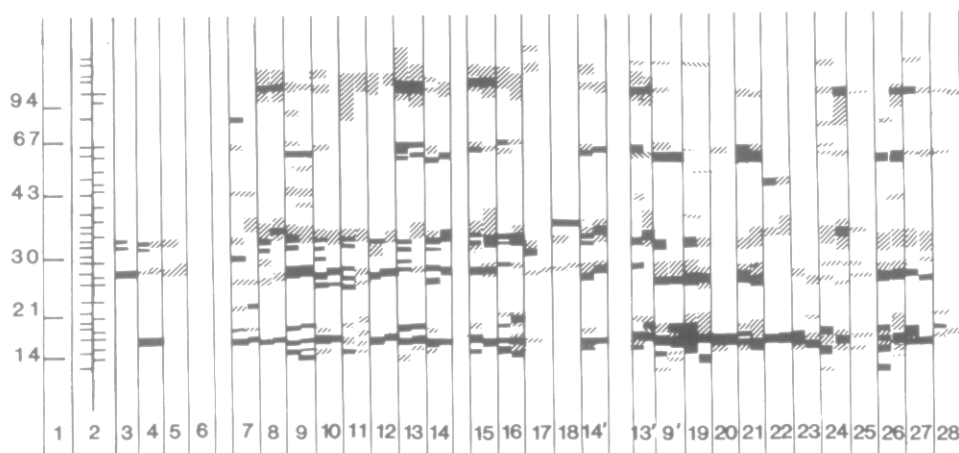


FIG. 3A. A comparison of the binding of the rabbit antisera specific for mite antigens and of IgE binding of individual sera to the NC strips from extracts M1 and C2 run on different halves of the same gel and presented in Fig. 2, A and B. The binding to the mite body extract is presented on the *left* side of each strip, and the mite culture extract on the *right* side. The binding was classified as either strong by a *black bar* or weak and/or diffuse by *hatching*. *Strip 1* illustrates the position of the MW markers in kilodaltons, and *strip 2* illustrates the position of the IgE-binding components in each extract. *Strips 3 through 28* are the same as for Fig. 2, A and B.

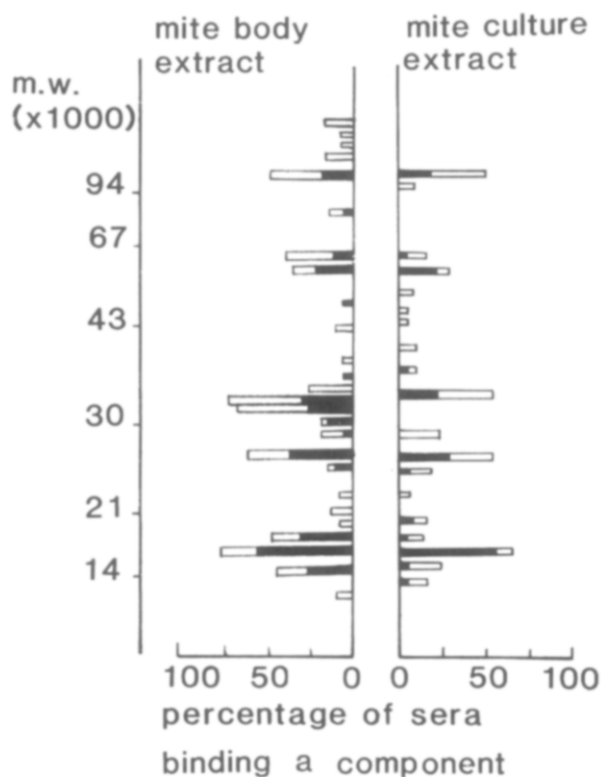


FIG. 3B. An analysis of the binding of sera from 22 mite-allergic subjects illustrated in Fig. 2, A and B and 3A. *Open boxes* illustrate the percentage of sera considered to bind weakly to a component, and *filled boxes* illustrate the percentage of sera considered to bind strongly to the mite body extract and mite culture extract components.

Several components were only detected in the mite body extract (MWs ~120, 110, 100, 70, 27, and 11 kd with the possible resolution of an additional 33 kd component from the 30 kd component). Of these components only the 27 kd component bound IgE strongly. The mite culture extract also contained components of MWs ~42 and 38 kd, which weakly bound IgE antibodies that were not detected in the mite body extract. As with the body extract, an additional component in mite culture extract of MW 89 kd could not be clearly resolved.

The densitometric scanning of the pairs of strips 11 to 14 in Fig. 2, A and B, are compared in Fig. 4. The MWs of the major IgE-binding components in both extracts and their distance of migration from the top of the gel are, in kilodaltons and centimeters, respectively, 95, 2.2; 53, 3.5; 25, 6.4; and 15, 8. The important IgE-binding component in mite body extracts of MW 30 kd and in culture extracts of MW 33 kd migrated a distance of 4.8 and 4.6 cm, respectively. In Fig. 4, *strip 11*, the binding to mite body components was greater than the binding to the corresponding component in the culture extract, whereas with *strips 12* and *14* the binding to the detected 15, 25, 30, 53, and 95 kd components was similar for each component in the two extracts. In *strip 13*, three of the components bound similar quantities of IgE, whereas with three other components, more IgE was bound to the mite body than the culture extract components of the same MW.

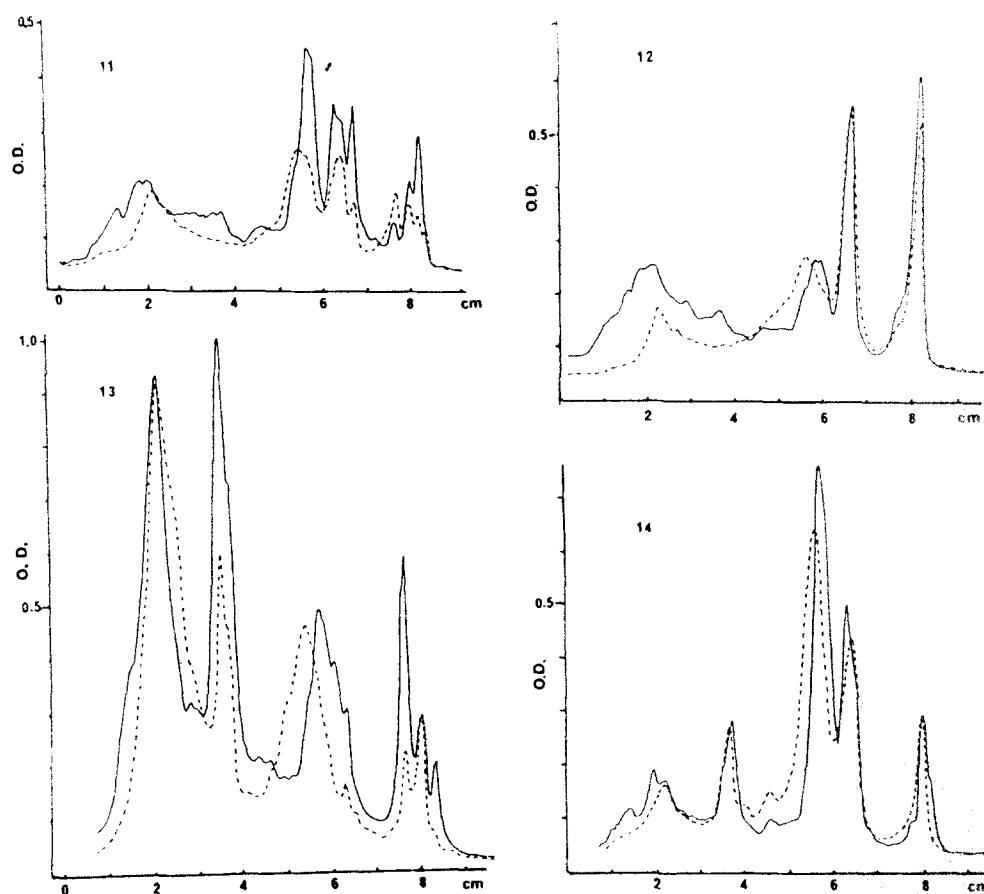


FIG. 4. A comparison of the relative quantity of specific IgE from individual sera binding to components of the same MW in extracts M1 and C2 by densitometric scanning of the autoradiographs of strips 11 through 14 presented in Fig. 2, A and B. Distance illustrated is from the top of the NC strip (i.e., high MW end).

Comparison of mite body and culture extracts as inhibitors and allergosorbents in RAST

When the mite body (M2) and culture (C1) extracts were each compared for their capacity to inhibit the binding of IgE to either extract on the solid phase, inhibition curves of different slope were produced (Fig. 5). With a culture extract on the solid phase (Fig. 5, A), both extracts produced almost parallel curves with the culture extract being more potent per unit of protein. When the mite body extract (Fig. 5, B) was used on the solid phase, the culture extract produced a relatively flatter inhibition curve, and the two extracts had similar potencies per unit of protein.

DISCUSSION

When mite body and culture extracts were transferred to NC and probed with mite-specific IgE, two features of the results were striking. First, overall differences between the extracts in the pattern of their

IgE-binding components detected by this technique were apparent. Some culture extracts had little capacity to bind specific IgE, whereas other extracts appeared to be deficient in one or more important IgE-binding components. Each of the mite body extracts examined demonstrated a similar range of IgE-binding components, although their relative proportions differed. Mite body extract freshly prepared by direct extraction from live mites demonstrated little IgE binding to the 30 and 15 kd components. The culture-type extracts differed more widely in their content of IgE-binding components, ranging from many components in extract C2 to few in extracts W and C1. The second striking feature was the disparity between the protein-staining pattern on the gels and the binding of IgE after transfer to NC and probing with a pooled serum from mite-allergic subjects. Although the higher MW components in the mite-culture extract C2 were only very weakly stained for protein, they were clearly detected by their IgE-binding after transfer to

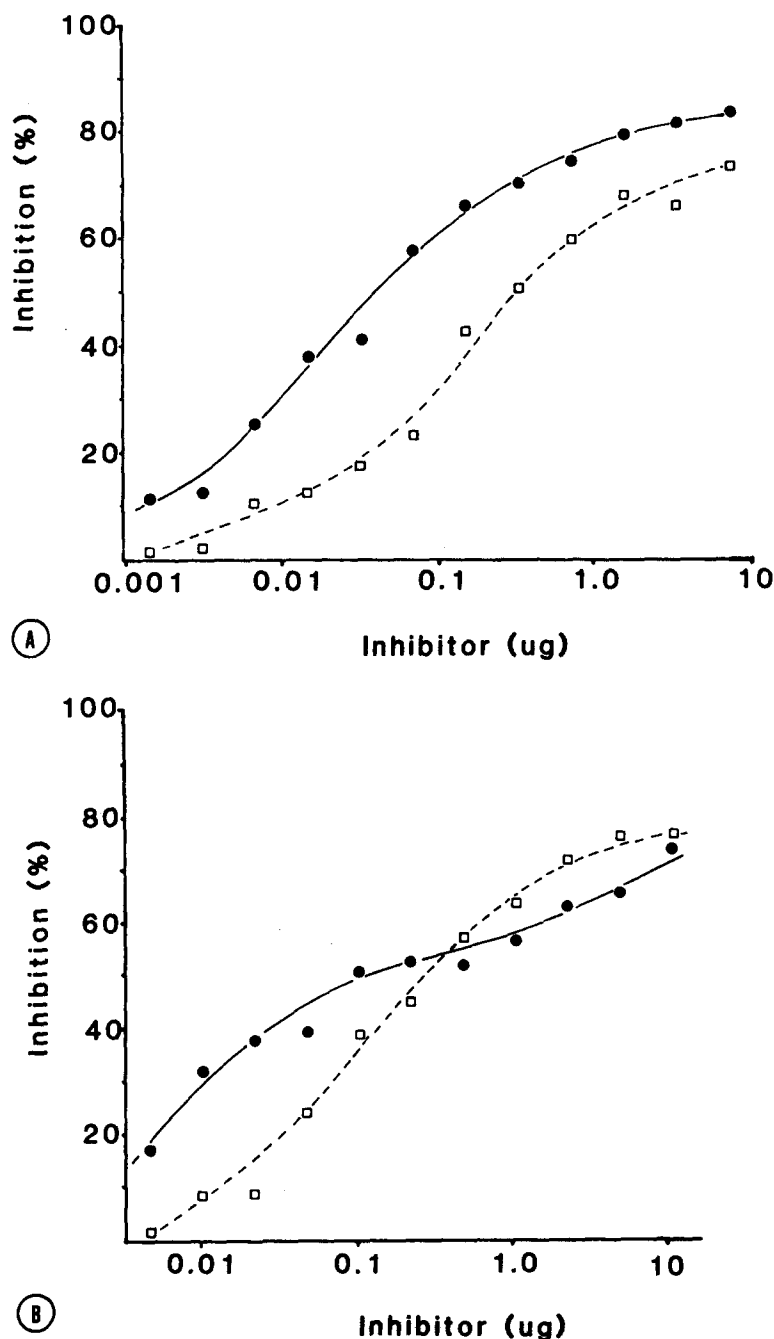


FIG. 5. A, Competitive RAST-inhibition assay with mite culture extract C1 bound to the solid phase and inhibition of mite-specific IgE binding by mite culture extract C1 (●) or by mite body extract M2 (□). **B,** Competitive RAST-inhibition assay with mite body extract M2 bound to the solid phase and inhibited with the same extracts as above.

NC. Detection was apparently highly sensitive, and probably much more material was added to the gel than was required to achieve labeling with IgE. The sensitivity of protein blotting has been observed by others.²⁴ Some components did not bind IgE on probing despite strong protein staining of the components on corresponding gels. The IS was relatively poorly

resolved by our SDS-PAGE system into six protein bands on a diffuse background, and on immunoprob- ing three or four IgE binding components were de- tected. These experiments were conducted three times with essentially the same result on each occasion. In one assay a faint 15 kd IgE-binding component was also detected (data not presented). Detection of low

MW components on protein blots may be less efficient because of the incomplete binding of these components on transfer to the NC²⁵ and later displacement of components from the NC²⁶ (and unpublished observations). In studies to establish the IS, 15 components were resolved on crossed immunoelectrophoresis, and four of these, plus two others not stained by CBB, bound IgE.¹⁴ The reasons for the discrepancy in detecting IgE-binding components between CRIE and protein blotting is not clear, particularly when other extracts were satisfactorily blotted. In our experiments the maximum practical quantity of IS was loaded on the gel (1.5 ampules per well).

A systemic study of the origins of the differences in allergen distribution between the different mite extracts is required. Not only were there differences in the source material for each extract, e.g., such as fecal-enriched culture, whole culture, live mites, and dried mites, but there were large differences in extraction times and in the duration and conditions of storage. Several of these extracts may be similar to extracts used clinically, and understanding the origin of these variations is important for optimizing the conditions to produce standardized mite extracts.

The mite body and culture extracts, M1 and C2, chosen for detailed comparison with individual sera, were considered to be the best available representatives of their type of extract in that they appeared to contain the maximum number of the important IgE-binding components. Comparison of the probing with many separate sera from mite-allergic subjects suggested that at least 16 common components (MWs 95, 58, 53, 40, 38, 35, 30, 26, 25, 22, 20, 18, 17, 15, 13, and 12 kd) were present in both extracts. These included the components that were the most strongly detected, and of the unpaired components, only the component of MW 27 kd in the mite body extracts was strongly bound by IgE. An IgE-binding component of this MW was evident in the culture extracts when they were probed with a pooled serum in Fig. 1.

Preparations of mite extract for clinical use should contain as many of the IgE-binding components as possible, although an extract containing the five most commonly and strongly bound components (MWs 95, 53, 32 to 28, 25, and 15 kd) would have detected mite allergy *per se* in 20 of the 22 sera tested. It is suggested that all mite extracts should contain these components.

In the 15 and 25 kd MW regions, in which allergens Der.p.II and Der.p.I, respectively, would be expected to be found, up to three IgE-binding components of only 1 or 2 kd difference in MW were detected, and no consistent pattern of IgE-binding to the different components in each region was observed. If only one

of these components is Der.p.I, then it emphasizes the difficulty of purifying this allergen to homogeneity by the reported methods.^{5, 27} Threefold differences in the measurement of Der.p.I in the same extract by four laboratories were attributed to probable differences in the internal Der.p.I. standards used.¹⁴ This would suggest that stringent criteria for purity of the mite allergens prepared with these MWs are required.

Except for allergens Der.p.I and Der.p.II, for which specific antisera and MW were available, it was not possible to associate the IgE-binding antigens recognized here with those recognized by CRIE.^{6, 8-11, 14}

Although the use of dissociating conditions was necessary to obtain efficient resolution of the antigens, direct RAST assays of the unfractionated mite extract have demonstrated that the capacity of mite allergens to bind IgE may be reduced by the SDS treatment.²⁸ It has been concluded, however, that despite initial dissociation by SDS, separated and transferred peptides on NC are usually capable of binding heterologous monospecific antisera.^{29, 30} In a study of rye grass-pollen allergens, Ford and Baldo³¹ detected seven previously described rye allergens on protein blots, in addition to eight other previously undescribed IgE-binding components.

The probing of transfers from three different gels with the same sera demonstrated that analysis by immunoblotting techniques is reproducible for both the minor and major components. However, the difficulty of analysis, particularly for components of slightly different MW, increases with the number of gels directly compared. Another limitation is that singly resolved components may represent two different antigens of the same MW. An advantage with NC-immunoprobe techniques, however, is that alternative and more detailed analysis of allergen source material by different one- and two-dimensional electrophoretic methods is feasible, including the use of isoelectric focusing and different buffers and detergents in gradient gels.

Densitometric scanning of autoradiographs allows the semiquantitative comparison of IgE binding to different regions of the same NC strip or to the same region of strips incubated with the different sera. Differences in the relative proportion of IgE bound to components of the same MW in the mite body and culture extracts by the different sera suggested further differences between these components, either at the level of loss or modification of determinants on individual components or in the number of components of this MW that were detected by the different specificities of the sera used.

This study clearly demonstrated the diversity of the IgE-binding components in a variety of mite extracts

derived from either mite bodies or from spent mite culture. Mite body extracts contained more allergens over a broad range of MW than did most of the culture extracts examined. The most restricted range of allergens was demonstrated by the IS. Several of the culture extracts were clearly deficient in important IgE-binding components, and until mite extracts do contain the full complement of such components, extracts prepared from mite bodies appear to provide a more reliable source of the important mite allergens. Presently, such extracts are used interchangeably for diagnosis, therapy, and the investigation of immune mechanisms, and it is likely that relevant allergens could be absent from some commercial extracts.

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