

Comparison of antigenic and allergenic composition of two partially purified extracts from *Dermatophagoïdes farinae* and *Dermatophagoïdes pteronyssinus* mite cultures

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Dermatophagoïdes farinae (Df 80d) and *Dermatophagoïdes pteronyssinus* (Dp 80d) extracts were analyzed for their antigenic and allergenic composition by means of crossed immunoelectrophoresis (CIE) and crossed radioimmunoelectrophoresis (CRIE). By CIE, 11 antigens could be numbered in Df 80d (Df 1 to Df 11) and seven antigens in Dp 80d (Dp 1 to Dp 7). This technique allowed us also to define antigens with common as well as specific parts for the two mite species. Among the antigens of *D. farinae* and *D. pteronyssinus*, only the antigen corresponding to Df 5 and Dp 5 seems to bear common epitopes to the two mite species, whereas Df 6 and Dp 4 appear to bear, respectively, specific epitopes of each species. Moreover, Df 11 appears to bear specific epitopes of *D. farinae*, although it shows a partial identity with Dp 7. By CRIE, on 20 mite-sensitive patients' sera, we identified, for each mite extract, the allergens responsive to human specific IgE. The allergograms show that the majority of mite-sensitive patients react with Df 11 and Df 6 and with Dp 7 and Dp 4. Thus, these antigens can be considered as major allergens. The minor allergens were also identified. None of these antigens was recognized by the control sera. Moreover, we observed that for one antigen (antigen 5) there exist antigenic determinants common to the two species of mites toward the rabbit serum and specific allergenic determinants to the human IgE response. A significant correlation was found between the specific IgE binding in CRIE and in RAST (Spearman coefficient: "rs" = 0.61 $p < 0.01$ for Df; "rs" = 0.78 $p < 0.01$ for Dp) (J ALLERGY CLIN IMMUNOL 71:588, 1983.)

The importance of the two acarians, *Dermatophagoïdes farinae* and *Dermatophagoïdes pteronyssinus*, in house-dust respiratory allergy has been recognized for a long time in many countries.¹⁻³ Extracts of these two mites have been studied through clinical investigations⁴⁻⁵ in asthmatic patients, and a close relationship between *D. farinae* and *D. pteronyssinus* has been found. Furthermore, laboratory experiments using conventional immunochemical and physico-chemical techniques⁶⁻⁸ have also been performed. They suggest that there is an antigenic cross-reactivity

between the two species and that the allergenicity is bound to proteinic fractions with a molecular weight around 10,000 to 50,000 daltons. Isolation and purification of these allergenic molecules were attempted, and an important allergen named P₁ has recently been isolated from a *D. pteronyssinus* extract.⁹

In our laboratory, we have previously studied the allergenic activity of partially purified *D. pteronyssinus* extracts¹⁰ and we have recently characterized and purified an important allergen, Df 11, from a partially purified *D. farinae* extract.^{11, 12}

In this work, we undertake a comparative immunological study between two partially purified extracts from *D. farinae* and *D. pteronyssinus* cultures, using CIE, CLIE, and CRIE. These methods were introduced and used by Scandinavian research workers to study pollens¹³ and animal hair¹⁴ allergens. The main advantage of the combination of CIE and CRIE meth-

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Received for publication Dec. 30, 1981.

Accepted for publication Jan. 11, 1983.

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Abbreviations used

CIE:	Crossed immunoelectrophoresis
CRIE:	Crossed radioimmunoelectrophoresis
CLIE:	Crossed-line immunoelectrophoresis
EDTA:	Ethylenediaminetetraacetic acid
RAST:	Radioallergosorbent test
PRIST:	Paper radioimmunosorbent test

ods is that allergens can be revealed without being previously isolated.

The aim of this study is to investigate the antigenic and allergenic composition of the partially purified extracts of *D. farinae* and *D. pteronyssinus*. Using CIE and CLIE in homologous or heterologous reactions between these two extracts and the respective rabbit antisera, we specify common and specific antigens for the two mite species.

Using CRIE method on 20 patients' sera sensitive to house-dust mite, we identify major and minor allergens from each mite extract. The results so obtained on the identification of some allergens could be considered as an important point for further purification work and for a better immunotherapy in house-dust allergy.

MATERIALS AND METHODS

Allergens

Partially purified extracts. The preparation of the partially purified extract from cultures of *D. farinae* and *D. pteronyssinus* mites was performed according to the method previously described in detail.^{10, 11} Each partially purified extract was dissolved in neutral distilled water and exhaustively dialyzed against the same medium until the optical density of the dialysate, at 280 nm, disappeared. The resulting brownish solutions have been named Df 80d for *D. farinae* and Dp 80d for *D. pteronyssinus*. They were lyophilized and constituted the experimental material.

Allergenic fractions from Df 80d. The S 75 fraction, particularly enriched in antigen 5 (Df 5) and antigen 6 (Df 6), and the DE fraction, which is the most purified form of the antigen 11 (Df 11), have been described in a preceding paper.¹²

Control. The control extract from the culture medium was treated by the protocol used to obtain the mite culture extract.

Antibodies

Antisera. With each extract, Df 80d or Dp 80d, immunization procedure was as follows. Three rabbits, weighing 2.5 kg, received 100 mg of the partially purified extract in 9 ml of physiological saline solution, mixed with the same volume of incomplete Freund adjuvant. The resulting emulsion was inoculated as follows: (1) subcutaneously at 10 points along the spine and (2) intraperitoneally (5 ml). After 1 mo, rabbits were boosted with 10 mg of the partially

purified extract diluted in 1 ml of physiological saline solution, by intravenous injection. This was repeated 1 mo later. One week after the last booster, blood samples were obtained. Sera were harvested, pooled, and concentrated four times by lyophilization.

Specific antibodies. To a determined volume of the pool of rabbit sera against Df 80d, a defined quantity of Dp 80d extract was added according to the equivalent point previously established on a quantitative precipitation curve. After 30 min at 20° C and 4 hr at 4° C, the mixture was centrifuged and the supernatant challenged in Ring test with increasing dilutions of Dp 80d extract. If a weak reaction appeared, a minute dose of Dp 80d was added and the process performed as described above until no reaction occurred in Ring test. Finally, specific antibodies against Df 80d, remaining in the supernatant, were concentrated by ammonium sulfate precipitation, at 50% of saturation; the precipitate so obtained was dissolved and exhaustively dialyzed against a physiological solution of NaCl. Aliquots of this solution were frozen and stocked for further use. This method has been applied as such to a pool of rabbit sera against Dp 80d using Df 80d extract for the preparation of specific antibodies against *D. pteronyssinus*. These concentrated specific antibodies were shown by CIE, to be totally depleted of antibodies having a cross-reactivity with the two mite species.

CIE methods

CIE as well as CLIE were performed as described by Weeke¹⁵ with some slight modifications. CIE was carried out on 7 by 10 cm plates (gel dimensions: 2 by 7 cm² and 3 by 7 cm² for the first dimension and the anodic antibody-containing gels, respectively). A 10 to 20 µl volume of each fraction was applied in the well. First- and second-dimension electrophoreses were performed under 20 V/cm for 30 min and 2 V/cm for 18 hr, respectively. The temperature of the cooling water was 15°. Specified amounts of rabbit antiserum were included in the second-dimension electrophoresis gel. Agarose gels were prepared as 1% solution in Tris-Veronal buffer, pH 8.7 (75 mM Tris, 24.5 mM Veronal, 0.63 mM calcium lactate, 0.2 mM sodium azide). After electrophoresis the gels were pressed, washed and pressed again, dried in hot air, and stained by Coomassie brilliant blue R 250. In the case of CLIE, intermediate gel measured 1 by 7 cm² and contained the allergenic fraction studied.

CRIE was performed as described by Weeke et al.¹³ with several modifications. After CIE (on 5 by 7 cm plates) the unstained gels dried in cold air were incubated overnight at room temperature with the 17-fold-diluted mite serum pool or individual sera. After washing, ¹²⁵I-labeled rabbit antiserum against IgE (kindly supplied by Pharmacia France), diluted in 8 ml of incubation buffer [50 mM phosphate buffer, pH 7.5, containing 0.3% bovine serum albumin, 150 mM sodium chloride, 15 mM sodium azide, 2.7 mM Na₂EDTA, 1% polysorbate 20 (Tween 20; ICI Americas, Inc., Wilmington, Del.)] to give 8 × 10⁴ cpm/ml in our gamma counter, was added. After an overnight incubation, the gels were washed, dried in cold air, and placed in an

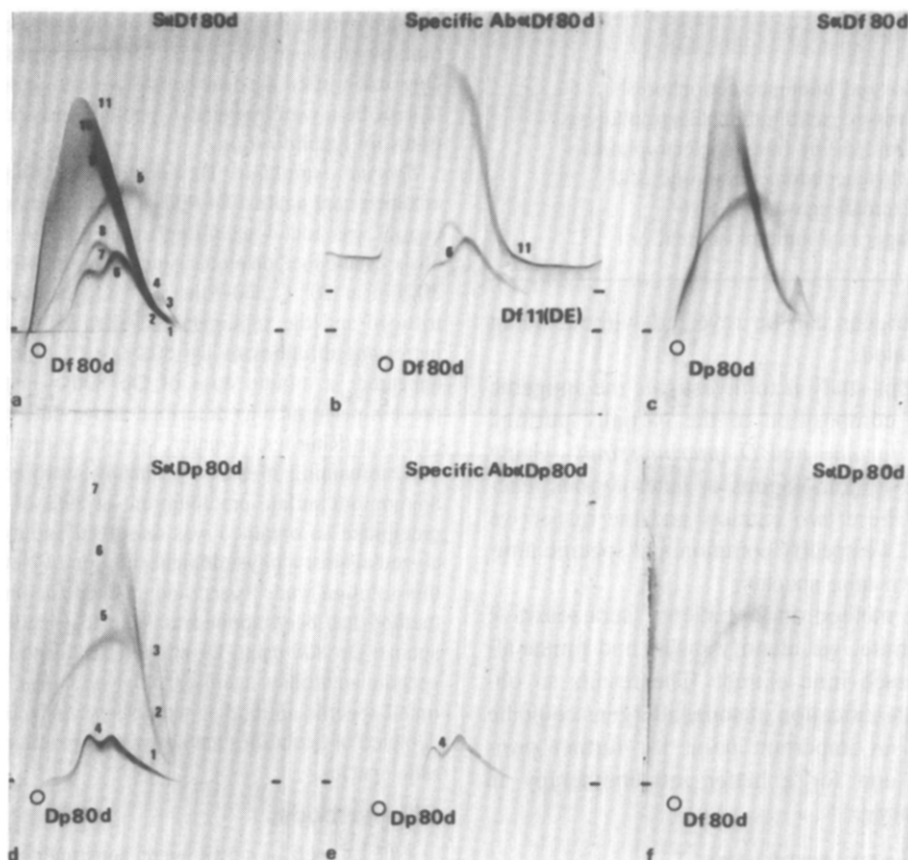


FIG. 1. **a**, CIE pattern of Df 80d reacting with rabbit anti-Df 80d serum. **b**, CLIE pattern of Df 80d reacting with specific rabbit antibodies against Df 80d; intermediate gel contains Df 11 (DE fraction). **c**, CIE pattern of Dp 80d reacting with rabbit anti-Df 80d serum. **d**, CIE pattern of Dp 80d reacting with rabbit anti-Dp 80d serum. **e**, CIE pattern of Dp 80d reacting with specific rabbit antibodies against Dp 80d. **f**, CIE pattern of Df 80d reacting with rabbit anti-Dp 80d serum. S α , Antiserum against; Ab α , antibody against; DE, most purified form of Df 11.

X-ray cassette covered with a Kodak X-O-Mat (Eastman Kodak Co., Rochester, N. Y.) film and an intensifier screen. They were exposed for several days at -20°C . A semiquantitative system was adopted to formulate the results according to the principle described by Löwenstein¹⁶: "The relative amount of specific IgE bound to the various precipitates was graded as the normalized reciprocal values of the corrected exposure times corresponding to specific visible radiostaining after a certain number of days." The choice of the exposure time was determined by comparing autoradiographs obtained with the mite reference serum and different control sera. In our system, 2, 5, and 10 days were considered, which correspond, respectively, to scores 100, 40, and 20. If a very strong radiostaining is observed at 2 days of exposure, the score was multiplied by factor 2. A visible radiostaining from 2 to 5 days was considered as a high degree of IgE binding, and a longer time as a low-affinity IgE binding. Finally, each glass gel was stained in a solution consisting of Coomassie brilliant blue R 250 in order to control the CIE pattern.

The results will be also illustrated by a histogram, called

allergogram, which shows the frequency of the reaction between specific IgE-containing patient sera and each allergen identified in the allergenic extract.

RAST

The partially purified extracts Df 80d or Dp 80d were coupled to CNBr-activated paper discs as described by Ceska et al.¹⁷ The RAST was performed according to the principle described by Wide et al.¹⁸ A 50 μl volume of diluted serum (1:17) was incubated with an allergen-coated paper disc during 3 hr at room temperature. The disc was washed, and 50 μl of ^{125}I -anti-IgE (Pharmacia France) was added. After an overnight incubation the disc was washed and counted in a gamma counter for bound radioactivity. The results were formulated as a percentage of bound radioactivity.

PRIST

The Phadebas test (Pharmacia, Uppsala, Sweden) was used for the determination of total IgE in the studied sera.

The levels of total IgE were quantified by the method of Ceska and Lundkvist.¹⁹ The results are reported as international units per milliliter.

Source of human sera

Sera from nondesensitized patients allergic to house-dust mite were used in this study. All patients were selected by the following criteria: allergic asthma and/or year-round rhinitis as clinical symptoms and positive skin tests to mite extracts (diameter of wheal ≥ 7 mm for mite extracts inoculated at 0.5 μ g/0.05 ml by intradermal route). All patients had specific IgE antibodies as determined by RAST (Classes 3 and 4). The mite reference serum pool was prepared as previously described.¹⁰ Cord serum, a pool of normal sera from nonallergic subjects, and a serum from a patient sensitive to *Dactylis glomerata* were used as control.

RESULTS

Preliminary CIE and CRIE experiments with *D. farinae* and *D. pteronyssinus* extracts failed to show cathodal protein bound in immunoprecipitates. All subsequent CIE and CRIE analyses were carried out with anodal antibody-containing gel.

Antigenic composition of *D. farinae* and *D. pteronyssinus* extracts

In CIE homologous reactions, 11 antigens (Df 1 to Df 11) can be numbered in Df 80d (Fig. 1, a), as previously shown,¹¹ and only seven antigens (Dp 1 to Dp 7) in Dp 80d (Fig. 1, d).

Using specific antibodies (as described in Material and Methods) for *D. farinae* and *D. pteronyssinus*, we determined antigens bearing specific epitopes for each of them. Fig. 1, b, clearly shows that Df 6 had specific epitopes of *D. farinae* as did Df 11, identified by CLIE using a purified form of Df 11. Fainter bands could be seen corresponding to Df 8, Df 3, and/or Df 4. Fig. 1, e, shows that Dp 4 had specific epitopes of *D. pteronyssinus*.

To determine antigens bearing common epitopes to *D. farinae* and *D. pteronyssinus*, we performed heterologous reactions. So, with Dp 80d and anti-Df 80d serum, five antigens were numbered as shown Fig. 1, c, and with Df 80d and anti-Dp 80d serum, four antigens were revealed (Fig. 1, f).

In order to identify some of these antigens bearing common epitopes to the two mite species, we used CLIE experiments.

In the Dp 80d-anti-Df 80d serum system, using purified antigen Df 11 in the intermediate gel, we have shown that there exists a partial fusion of Df 11 with a line corresponding to Dp 7 (Fig. 2, a). This antigen has been identified as Dp 7, by means of antigen 42 of *D. pteronyssinus* (kindly provided by H. Löwenstein) that was shown to be equivalent to Dp 7

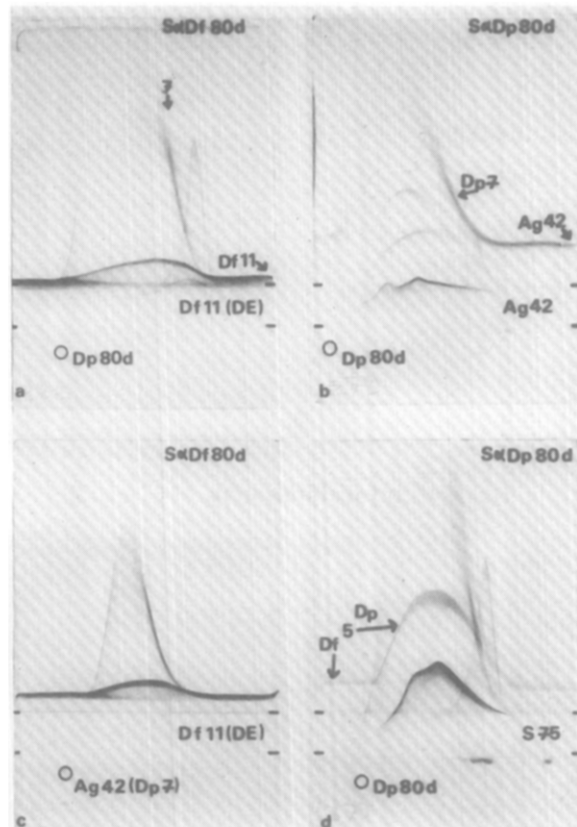


FIG. 2. a, CLIE pattern of Dp 80d reacting against rabbit anti-Df 80d serum; intermediate gel contains Df 11 (DE fraction). b, CLIE pattern of Dp 80d reacting with rabbit antiserum against Dp 80d; intermediate gel contains Ag 42. c, CLIE pattern of Ag 42 reacting with rabbit anti-Df 80d; intermediate gel contains Df 11. d, CLIE pattern of Dp 80d reacting with rabbit anti-Dp 80d serum; intermediate gel contains S 75 fraction (Df 5 and Df 6). Ag, Antigen; other abbreviations as in Fig. 1.

(Fig. 2, b). Thus we see (Fig. 2, c) that the pattern in antigen 42 (Dp 7)-anti-Df 80d serum system using Df 11 in the intermediate gel, confirmed that Df 11 and Dp 7 bore common epitopes and that Df 11 had specific epitope of *D. farinae*.

Using the S 75 fraction from Df 80d, which is known to contain mainly antigens Df 5 and Df 6,¹² we have identified by CLIE (Fig. 2, d) one of the four common antigens revealed (Fig. 1, f) as probably being antigen 5, if we assume that Df 6 is specific of *D. farinae* as stated above (Fig. 1, b).

Culture medium extracts did not show any immunoprecipitate in CIE.

Allergenic composition of Df 80d and Dp 80d

The two mite extracts were successively analyzed by the CRIE technique with 20 individual sera from mite-sensitive patients.

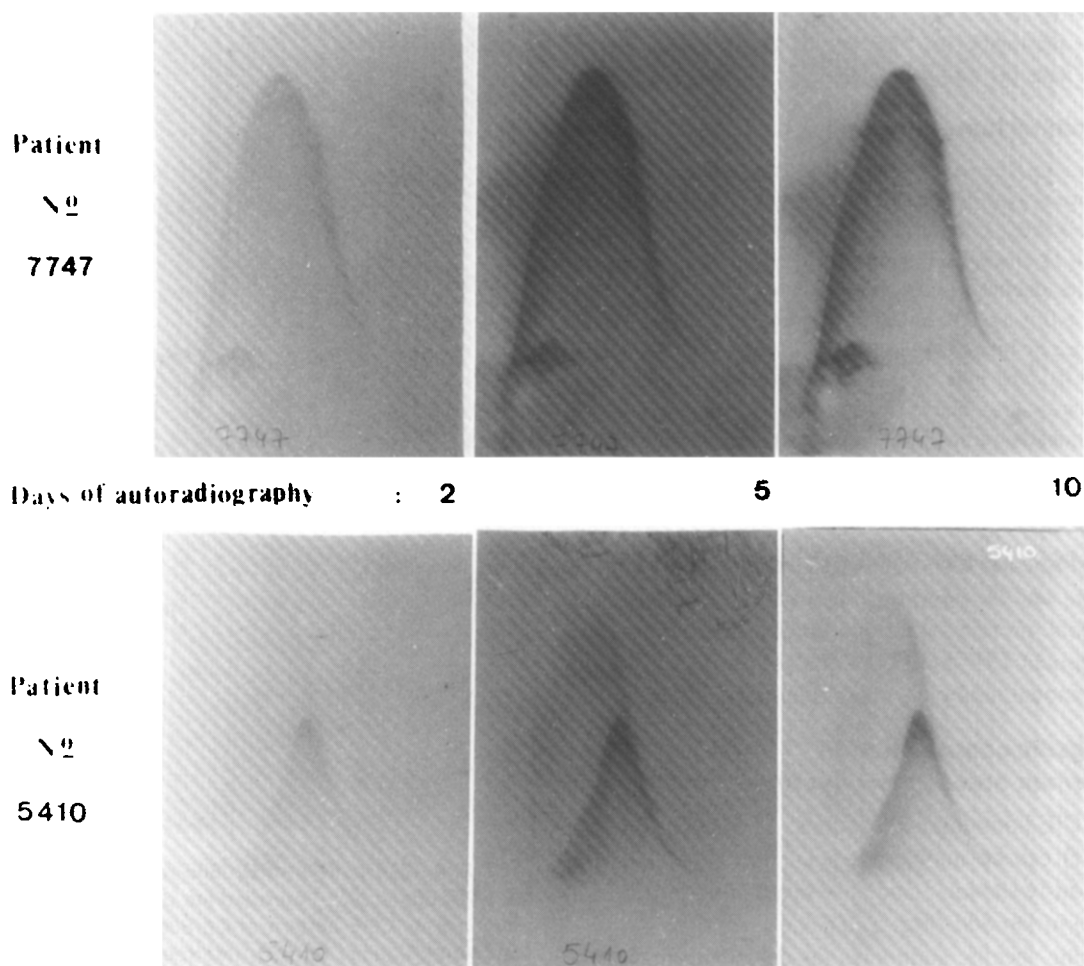


FIG. 3. Autoradiographs of the CIE pattern of *D. farinae* extract (Df 80d) with human sera (patients 7747 and 5410).

The specificity of IgE binding was carefully controlled by testing serum pool from nonallergic subjects and a serum from a pollen-sensitive patient. After 10 days of exposure time, these different controls showed a weak radiostaining, with Df 80d extract considered as unspecific. Consequently, radiostaining stronger than the control obtained at 10 days was considered specific, but with a low-affinity IgE binding.

In Fig. 3, we can observe the kinetic of the radiostaining in the immunoprecipitates of Df 80d after addition of sera from patient 7747 and patient 5410 containing anti-mite specific IgE revealed by means of ^{125}I -anti-IgE.

With serum 7747, we saw only one radiolabeled immunoprecipitate corresponding to antigen 11 (Df 11). This one appeared on the second day. For serum 5410, only Df 6 was radiostained on the second day, Df 11 appeared on the fifth day, and Df 3 and Df 4 appeared on the tenth day.

Fig. 4 shows an example of CRIE experiments using Dp 80d and the same sera, 7747 and 5410.

For serum 7747, only Dp 7 appeared on the second day, Dp 4 was radiostained on the fifth day, and nothing else appeared on the tenth day. With serum 5410, the sequence was Dp 4 on the second day, Dp 7 on the fifth day, and Dp 6 on the tenth day.

From the autoradiographs, according to the semi-quantitative system as defined in Material and Methods, a CRIE score was assigned to the individual radiolabeled immunoprecipitates revealed by each of the sera examined. We can see in Table I the detail of these results for 20 mite-sensitive patients. All (100%) of the sera contained specific IgE which highly bound with Df 11 from Df 80d; however, only 11 sera (55%) had specific IgE against Df 6, and some of them had IgE against Df 3, 4, 5, and 8. These results are shown in the allergogram in Fig. 5. When the antigens from *D. pteronyssinus* were used to study the same sera, the following results were obtained:

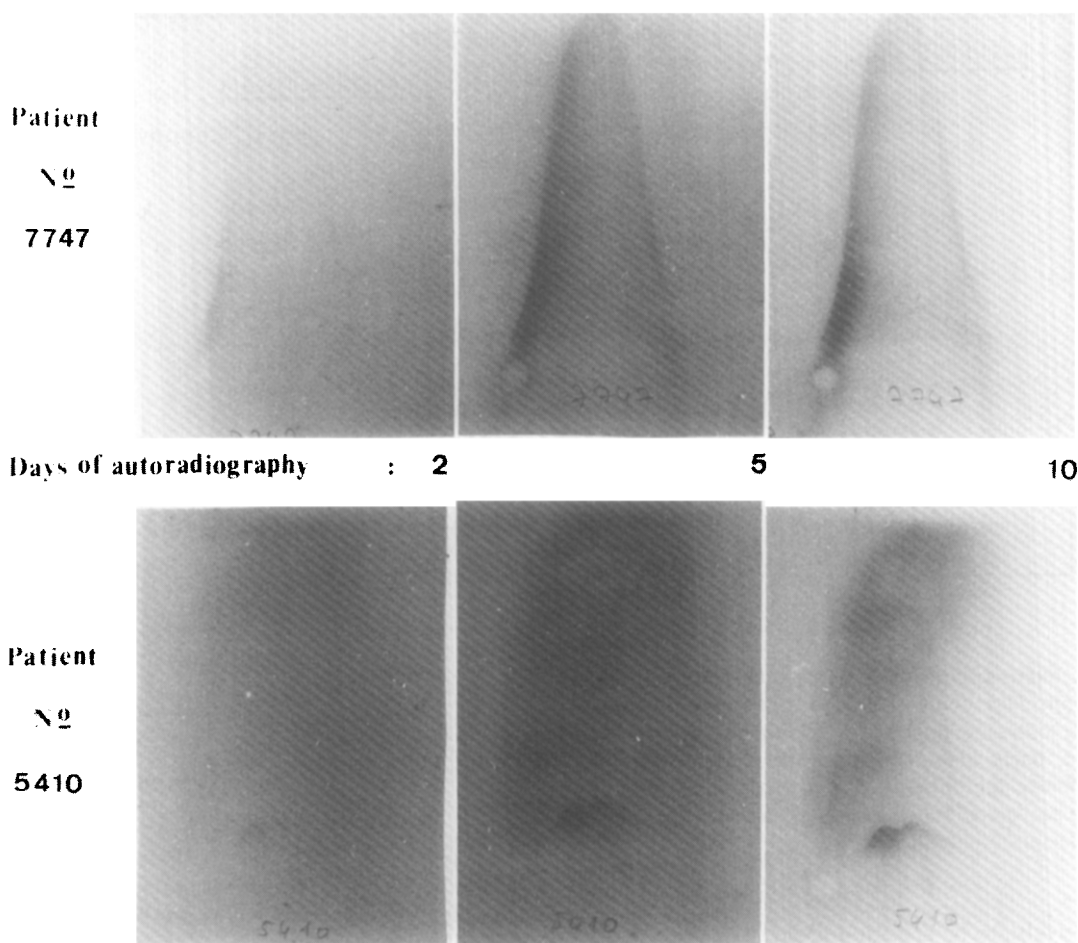


FIG. 4. Autoradiographs of the CIE pattern of *D. pteronyssinus* extract (Dp 80d) with human sera (patients 7747 and 5410).

100% of the sera contained specific IgE which highly bound with Dp 7, 85% to Dp 4, and a few sera to Dp 3, 5, and 6. The results are shown by the allergogram of Fig. 6.

Statistical correlations between CRIE, RAST, and PRIST

The results concerning the evaluation of specific IgE by RAST and the determination of total IgE by PRIST from the 20 mite-sensitive patients' sera are given in Table I.

We found a significant positive correlation between RAST expressed in percent of bound radioactivity and CRIE expressed in total score for each mite extract. The Spearman coefficient ("rs") was as follows: For Df 80d $rs = 0.61$, $p < 0.01$; for Dp 80d $rs = 0.78$, $p < 0.01$.

When one compared total serum IgE in international units per milliliter and CRIE score for Df 80d and Dp 80d, there was no correlation ($rs < 0.45$ for each mite).

DISCUSSION

In this work, we report a comparative study between antigens and allergens Df 80d and Dp 80d, partially purified mite culture extracts, using CIE, CLIE, and CRIE. Some antigenic relationship between the two mite species was suggested by Biliotti et al.,⁸ using rabbit antisera in agarose gel double diffusion (Ouchterlony method), but Lind²⁰ proved the value of the CIE method to detect mite antigens and allergens in extracts from different sources.

First, results obtained by CIE suggest that, among the 11 antigens of Df 80d and the seven antigens of Dp 80d, antigens with common as well specific parts do exist for the two mite species. All attempts failed to show a reaction between an extract obtained from the culture medium and the antisera against Df 80d and Dp 80d.

Among the antigens of *D. farinae* and *D. pteronyssinus*, only the antigen corresponding to Df 5 and Dp 5 seems to be common to the two mite species, as shown in Fig. 2, *d*, whereas Df 6 and Dp 4 appear,

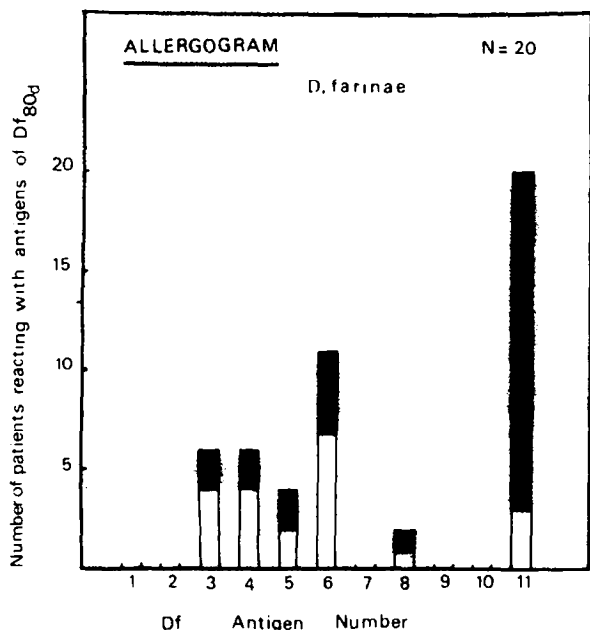


FIG. 5. Allergogram for antigens of Df 80d based upon sera from 20 patients allergic to mites. The specific IgE binding to the individuals antigens was obtained by CRIE method. *Black area*, Time-dependent radiostaining for high-affinity IgE binding (CRIE score = 200, 100, 40); *white area*, low-affinity IgE binding (CRIE score = 20). See Materials and Methods.

respectively, to be specific for the two species shown in Fig. 1, *b* and *e*.

In addition to the antigens common to the two species (Dp 5 and Df 5) and the specific antigens for each of them (Df 6 and Dp 4), it must be noted that Df 11 appears to bear specific epitopes of *D. farinae* when reacting with specific antibodies against *D. farinae* (Fig. 1, *b*) whereas it shows a partial identity with Dp 7 in CLIE (Fig. 2, *a* and *c*) when both of them react with the antiserum against Df 80d.

Second, among the several antigens revealed by CIE, we attempted to find the allergens specifically reactive to the IgE that they have induced in the patients. Thus CRIE experiments performed with 20 sera from mite-sensitive patients allowed us to identify allergens in the *D. farinae* and *D. pteronyssinus* extracts.

The value of the CRIE technique has been confirmed by a comparative study with the RAST, and significant correlation was found between the RAST and the CRIE methods in our analysis of the two mite extracts. Other authors have observed the same correlation when analyzing a group of patients sensitive to timothy¹³ and to cow dander.²¹ Another advantage of this technique is to individually characterize one allergen or more and

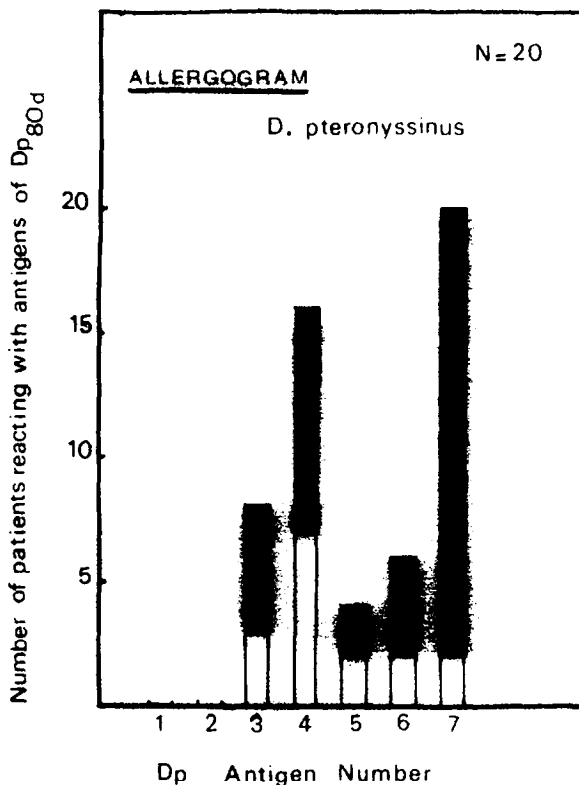


FIG. 6. Allergogram for antigens of Dp 80d, analyzed by CRIE as in Fig. 5. *Black area*, Time-dependent radiostaining for high IgE binding (CRIE score = 200, 100, 40); *white area*, low IgE binding (CRIE score = 20). See Materials and Methods.

to evaluate the frequency of the human IgE response vs. one particular allergen.

We can observe that all patients who react with Df 11 also react with Dp 7. This double allergenic activity can be explained either by a sensitization to one of these antigens bearing common epitopes to the two mite species, or to both of these antigens. The first explanation is supported by our results concerning the antigenic composition of the two mite extracts as determined in CIE using rabbit sera, particularly for Df 11 and Dp 7 as mentioned above.

We can also notice that some sera that contain specific IgE against Df 6 do not react with Dp 4, and vice versa (Figs. 3 and 4). These results suggest that Df 6 and Dp 4, major allergens as stated by allergograms (Figs. 5 and 6, respectively), bear specific epitopes. This was also demonstrated through the antigenic analysis of Df 80d and Dp 80d (Fig. 1).

By contrast, antigen 5 that appeared as common to *D. farinae* and *D. pteronyssinus*, when reacting with rabbit antiserum, shows a specificity for each species when they are recognized in human patient sera. This

TABLE I. Data about 20 patients allergic to mites and three control persons

Patient No.	RAST (% of bound radioactivity)		PRIST (IU/ml)	Individual antigens binding IgE (CRIE score as superscript)			Total CRIE score	
	Df 80d	Dp 80d		Df 80d	Dp 80d		Df 80d	Dp 80d
1	1.9	2.6	45	11 ⁴⁰	7 ¹⁰⁰ 6 ²⁰ 5 ²⁰ 4 ⁴⁰		40	180
2	2.8	2.3	110	11 ²⁰	7 ²⁰		20	20
3	3.1	3.7	840	11 ¹⁰⁰ 6 ²⁰	7 ¹⁰⁰ 4 ²⁰		120	120
4	3.3	3.7	140	11 ¹⁰⁰ 4 ²⁰ 3 ²⁰	7 ¹⁰⁰ 5 ²⁰ 4 ²⁰		140	140
5	4.8	4.4	210	11 ¹⁰⁰	7 ⁴⁰ 4 ⁴⁰		100	80
6	6.7	4.9	173	11 ⁴⁰ 6 ²⁰ 5 ²⁰	7 ²⁰		80	20
7	6.9	11	245	11 ¹⁰⁰	7 ¹⁰⁰ 4 ⁴⁰		100	140
8	7.8	7.3	640	11 ¹⁰⁰ 6 ¹⁰⁰ 4 ⁴⁰ 3 ⁴⁰	7 ¹⁰⁰ 4 ²⁰		280	120
9	8	2.7	940	11 ¹⁰⁰ 6 ²⁰	7 ¹⁰⁰ 4 ²⁰		220	120
10	9.5	9.7	145	11 ¹⁰⁰	7 ¹⁰⁰ 4 ²⁰ 3 ²⁰		100	140
11	11.4	21	900	11 ¹⁰⁰ 8 ²⁰ 6 ⁴⁰ 5 ⁴⁰	7 ²⁰⁰ 6 ⁴⁰ 3 ⁴⁰		200	280
12	12.6	14.1	320	11 ¹⁰⁰ 4 ²⁰ 3 ²⁰	7 ¹⁰⁰ 6 ⁴⁰ 3 ⁴⁰		140	180
13	12.6	14.6	260	11 ¹⁰⁰ 6 ²⁰ 4 ²⁰ 3 ²⁰	7 ¹⁰⁰ 4 ²⁰ 3 ²⁰		160	140
14	14	13.1	180	11 ¹⁰⁰ 6 ¹⁰⁰ 5 ²⁰	7 ⁴⁰ 4 ²⁰		220	60
15	17.5	17.1	500	11 ¹⁰⁰ 8 ¹⁰⁰ 6 ¹⁰⁰ 5 ⁴⁰	7 ¹⁰⁰ 6 ¹⁰⁰ 5 ¹⁰⁰ 4 ²⁰⁰ 3 ⁴⁰		340	540
16	17.9	14.3	600	11 ¹⁰⁰ 6 ²⁰⁰ 4 ⁴⁰ 3 ⁴⁰	7 ⁴⁰ 6 ²⁰ 4 ²⁰⁰		380	260
17	17.6	24.5	350	11 ¹⁰⁰ 6 ²⁰	7 ²⁰⁰ 6 ²⁰⁰ 5 ¹⁰⁰ 4 ¹⁰⁰ 3 ⁴⁰		120	640
18	17.9	13.1	200	11 ¹⁰⁰	7 ¹⁰⁰ 4 ⁴⁰		100	140
19	20.3	22.6	560	11 ²⁰⁰	7 ²⁰⁰ 4 ²⁰⁰ 3 ⁴⁰		200	440
20	25.1	21.7	1600	11 ²⁰⁰ 6 ²⁰ 4 ²⁰ 3 ²⁰	7 ²⁰⁰ 4 ²⁰⁰ 3 ²⁰		260	420
Cord serum	0.4	0.6	20					
Nonallergic sera	0.3	0.3	55					
Pollen-allergic patient	0.6	0.5	1800					

suggests that if the antigen 5 of each species possesses a great number of common antigenic determinants, each one certainly has specific allergenic determinants recognized by the human immune system.

Drawing from these results, we have shown that the CIE and CRIE techniques not only permit the analysis of the antigenic and allergenic composition of an allergenic extract but also reveal the individual sensitivity of an allergic subject toward one or another allergenic molecule. Furthermore, the fact that, for one antigen, there exist antigenic markers common to the two species of mites toward the rabbit serum and specific allergenic determinants to the human IgE response demonstrates the complexity of immunologic and allergic phenomena induced by allergens.

The help of Mrs. G. Allain for typing the manuscript is acknowledged.

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