

# Demonstration of Species-Specific Sensitization to Major Allergens of *Dermatophagoides* Species by Solid-Phase Absorption of Human IgE Antibodies

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Twenty-five mite-allergic patients in the Brussels area were tested with the skin-prick test (1, 5 and 10 histamine equivalents by prick test (HEP)) and RAST with standardized extracts of *Dermatophagoides pteronyssinus* (Dp) and *D. microceras* (Dm). Highly significant correlations ( $0.69 < r_s < 0.96$ ) between the two species coincided with marginal, but highly significant ( $P < 0.01$ ), greater reactions to Dp in all comparisons. Species-specific IgE antibodies to antigen Dp-42 (=P1) and Dp-X were readily detected by solid-phase serum absorption with heterologous antigen and subsequent crossed radioimmuno-electrophoresis (CRIE). An absorption single radial immunodiffusion (SRID) technique, involving major allergens Dp-42, Dm-6 and Df-6 of Dp, Dm and *D. farinae* (Df), demonstrated the presence of IgE antibodies reacting with (1) epitopes common to all species, (2) epitopes common to two species, absent on the third, and (3) epitopes strictly specific for Dp. The majority of patients with a strong IgE binding reacted to both common and strictly species-specific epitopes. The serological findings were in good agreement with the exposure situation in the area; by species-specific ELISA for dust allergen content, Dp occurred as the dominating species, 20 out of 24 homes having dust levels of Dp-42 above 30 ng/g. Five and four homes had detectable levels of Dm-6 and Df-6, respectively, in dust.

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Exposure to house dust mites of the family *Pyroglyphidae* is a frequent cause of IgE-mediated perennial asthma and rhinitis [16, 23]. Species of the genus *Dermatophagoides*, *D. pteronyssinus* (Dp) and members of the *D. farinae* group (*D. farinae* (Df) and *D. microceras* (Dm)), both occur worldwide [3], and it has recently been demonstrated that *D. microceras* occurs with a high frequency in northern Europe [11]. Like the situation in the case of allergy to pollens of grasses and deciduous trees and to venoms of wasps (*Vespidae*), sensitization thus takes place to one or more of a number of closely related species, depending on their prevalence in the patients' environment.

Species-specific as well as common antibody

responses to major allergen P1 (=Dp-42) of Dp and corresponding allergens Dm-6 and Df-6 were recently demonstrated in rabbits [10] and mice [4, 5]. In the present study, we demonstrate evidence of species-specific patient reactions to standardized extracts in common diagnostic tests, like the skin-prick test and RAST, findings that are confirmed and extended by studies of the IgE response of absorbed sera to well-defined major allergens.

## MATERIALS AND METHODS

*Patients.* Twenty-five residents (aged 7–51 years) in the Brussels area, with perennial asthma ( $n=4$ ),

rhinoconjunctivitis ( $n=15$ ), or both ( $n=6$ ) associated with exposure to house dust and house dust mites, and with positive skin-prick test ( $n=5$ ), RAST ( $n=1$ ), or both ( $n=18$ ) to *D. pteronyssinus* were selected for the study. During an ordinary surgery visit, the patients were skin-prick tested, a blood sample was taken for specific IgE determination and instructions were given to collect a dust sample from the subject's home.

Normal serum was a pool from 1000 healthy blood donors.

**Dust samples.** Sampling of dust from 24 homes (two patients were members of the same family) took place by vacuum-cleaning mattresses, bedroom floors, and living room floors once during the months March–June.

**Antigen/allergen preparations.** Reference allergen preparations of the three *Dermatophagoides* species Dp, Dm and Df were dialysed and freeze-dried extracts of whole mite cultures (WMC) were made as previously described [12]. The species identity and homogeneity of the cultures was assured by microscopy as in [10].

Acetone-precipitated mite excrement antigens (Dp-AML, Dm-AML and Df-AML) were produced as described in [12].

Purified major excrement allergens, Dp-42 (=P1), Dm-6 and Df-6 for use in IgE absorption experiments had previously been characterized [9, 10].

Purified major allergen, Dp-X, and oligospecific antibodies to Dp-X, employed to establish the identity of Dm-X on the CRIE immunoplates and autoradiographs, had previously been characterized [9].

Preparations of Dp and Dm for skin-prick testing were standardized extracts (Soluprick SQ, ALK, Copenhagen, Denmark). Extracts were supplied in 50% glycerol in concentrations of 1, 5 and 10 HEP (histamine equivalents by prick test). The content of the major allergens Dp-42 and Dm-6 were 1.7  $\mu\text{g}/\text{ml}$  and 2.0  $\mu\text{g}/\text{ml}$ , respectively, at 1 HEP, according to the manufacturer's specifications.

**Antibody reagents.** Polyspecific rabbit antibodies to DP reference extract and monospecific rabbit antibodies to allergens Dp-42, Dm-6 and Df-6 were the same as previously applied [10, 12]. Radiolabelled ( $^{125}\text{I}$ ) rabbit anti-human IgE [14] was a gift from Dr H. Löwenstein, the Protein Laboratory.

**ELISA for dust concentration of mite antigen.** The dust bags were sliced open and the recovered dust content of each bag was weighed. Extraction took place 1:2 (wt/vol) in 0.125 M ammonium hydrogen carbonate for 3 h at room temperature. The ELISA procedure for the measurement of Dp-42, Df-6 and Dm-6 is described elsewhere [11]. In short, 100  $\mu\text{l}$  dust extracts and dilutions (1:10 and 1:100) were transferred to wells in polystyrene microtitre trays previously coated with affinity-purified, monospecific rabbit antibodies to major allergens Dp-42, Df-6 and Dm-6. After overnight incubation and repeated washings, horseradish peroxidase-conjugated rabbit antibodies to allergens Dp-42, Df-6 and Dm-6, rendered species-specific by Sepharose column immunoabsorption, were added. After a 1-h incubation period and repeated washings, substrate (hydrogen peroxide/orthophenylenediamine) was added to each

well and the reaction was carried out for 15 min in darkness. Reading of the absorption at 492 nm took place 5 min after the addition of 1.0 M sulphuric acid. Standard response curves were constructed from reference whole mite culture extract solutions of each species with major allergen concentrations in the range 1.2–600 ng/ml. Assay sensitivity was 8, 3 and 30 ng/ml for allergens Dp-42, Df-6 and Dm-6, respectively.

**Skin-prick test.** The skin-prick test was carried out as described in [26] using histamine hydrochloride, 1 mg/ml, as the positive reference and saline as the negative control. The skin index was calculated as wheal area for extract divided by histamine wheal area.

**RAST.** Specific serum IgE by RAST to Dp and Dm was carried out as described in [25], using paper discs coupled with reference extracts, Dp-WMC and Dm-WMC (25 discs/ml, 1 mg WMC/ml). Results are given as retained counts in percentage of total added radioactivity.

**Gel-precipitation techniques.** Crossed immunoelectrophoresis (CIE) and modifications thereof (crossed-line immunoelectrophoresis and CIE with antibodies in the intermediate gel) were carried out as described in [13]. Single radial immunodiffusion (SRID) was carried out essentially as in [15].

**Immunoabsorption of patient sera for crossed radio-immunoelectrophoresis (CRIE).** Patient serum (650  $\mu\text{l}$ ) was applied to a column containing 0.5 ml protein A-Sepharose (Pharmacia, Uppsala, Sweden), equilibrated in 0.1 M Tris/acetate buffer, pH 7.7. A further 650  $\mu\text{l}$  buffer was applied. The total eluate was divided into 3  $\times$  400  $\mu\text{l}$  aliquots and applied to the following columns: 2  $\times$  1 ml CNBr-activated Sepharose 4B, coupled with (1) 4 mg Dp-AML, (2) 4 mg Dm-AML, and (3) 1 ml Sepharose 4B (control column). The columns were equilibrated in 0.07 M phosphate buffer, pH 7.0, and elution took place with 3  $\times$  1.5 ml of the same buffer, followed by 3  $\times$  0.75 ml phosphate buffer, containing 0.5 M sodium chloride. All eluted fractions were pooled for incubation. Bound antibodies were not eluted.

**Immunoabsorption of patient sera for absorption SRID.** Patient serum (4  $\times$  100  $\mu\text{l}$ ) was applied to three columns containing 0.5 ml CNBr-activated Sepharose 4B, coupled with 2 mg Dp-AML, Dm-AML and Df-AML, respectively, and to one column containing 0.5 ml Sepharose 4B (control column). The columns were equilibrated as above and eluted with 2  $\times$  0.5 ml phosphate buffer and 3  $\times$  0.25 ml phosphate buffer containing 0.5 M sodium chloride.

**Detection of allergen-specific IgE for absorption CRIE.** CRIE was carried out essentially as previously described [24]. CIE plates (7  $\times$  5 cm) with 15  $\mu\text{g}$  Dp-AML and 15  $\mu\text{g}$  Dm-AML applied separately and electrophorized into 150  $\mu\text{l}$  rabbit anti Dp-WMC were incubated overnight with serum eluate from immunoabsorption columns, adjusted to 8 ml with 0.07 M phosphate buffer, pH 7.0. After washing, incubation with 8 ml labelled anti-IgE (a total of 225,000 cpm in our gamma counter) took place overnight. Washed and dried plates were autoradiographed for 10 days at  $-20^\circ\text{C}$  with intensifying screens. The radiostaining

intensity was scored by comparison with simultaneously performed autoradiographs of plates with two-fold dilutions of the labelled reagent applied in SRID against swine anti-rabbit IgG (Dakopatts, Copenhagen, Denmark) [27]. Thus a number of CRIE classes, with two-fold increasing scores, was obtained. The position of antigens Dp-42, Dp-X, Dm-6 and Dm-X was identified by CLIE with purified antigens and CIE with antibodies to Dp-42 and Dp-X in the intermediate gel.

**Detection of allergen-specific IgE for absorption SRID.** Purified antigens Dp-42, Dm-6 and Df-6 (0.2, 0.16 and 0.4  $\mu\text{g}$ , respectively) were separately applied for SRID, using a mixture of monospecific antibodies to Dp-42, Dm-6 and Df-6 (0.1, 0.05, and 0.05  $\mu\text{l}/\text{cm}$ , respectively). Applied amounts of antigen and antibody were adjusted so as to give the same precipitate diameter with an approximately even antigen weight. The antibody-containing agarose gel was supported by agarose-coated plastic sheets (Gelbond, Marine Colloids, FMC, Rockland, Maine). After diffusion for 2 days at 20°C, the gel was pressed, washed, and dried, and supported gel strips were cut out (15×50 mm), each containing circular precipitates (9–10 mm diameter) of the three allergens investigated. Each gel strip was incubated overnight with serum eluate from immunoabsorption columns, adjusted to 4 ml with the phosphate buffer. Incubation with 4 ml labelled anti-IgE (120,000 cpm) took place as above and was followed by autoradiography for 7 days at -80°C. The radiostaining intensity was scored as described above.

**Statistics.** Nonparametric statistical tests (Spearman rank correlation and Wilcoxon matched-pairs signed ranks test) was performed according to Siegel [18].

## RESULTS

Measurement of dust allergen levels demonstrated a clear dominance of Dp (Fig. 1); 20 out of 24 homes had levels of Dp-42 above 30 ng/g and a maximum value of 7800 ng/g. Dm and Df were detected in five and four homes, respectively. Two dust samples (nos 1 and 20) contained high levels of Dm (>7000 ng/g). The median skin index at 1 HEP was close to unity

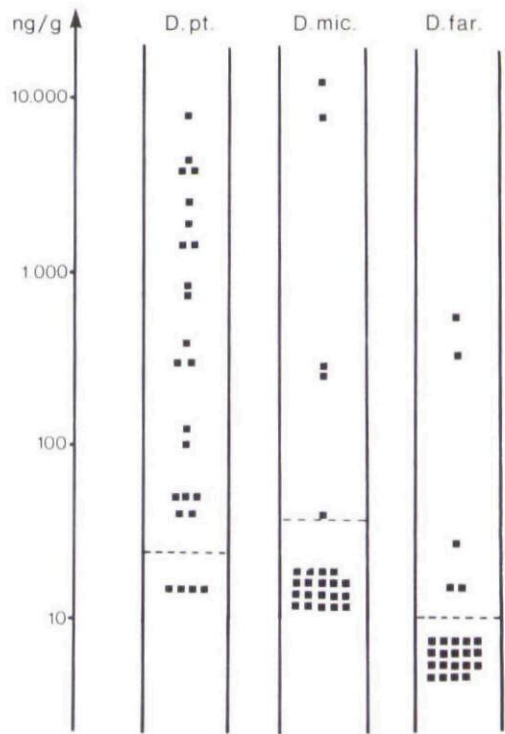


Fig. 1. Dust levels of major allergens Dp-42, Dm-6 and Df-6 in 24 Belgian homes, as determined by species specific ELISA. Vertical axis: major allergen concentration in ng/g dust. Broken horizontal lines indicate limits of detection.

(1.12 for Dp and 0.88 for Dm), in good accordance with the manufacturer's HEP declaration (Table 1). Highly significant correlations between reactions to the two species were obtained for both the skin-prick test and RAST (Table 1, Fig. 2). At all tested extract concentrations (1, 5, and 10 HEP), however, the mean response to Dp in the skin-prick test was significantly greater than to Dm, when tested by the

TABLE 1. Median values of skin-prick test and RAST with allergen extracts of *D. pteronyssinus* and *D. microceras*. Spearman rank correlation test and Wilcoxon matched-pairs signed-ranks test

|                         |    | Skin-prick test* at |        |        | RAST (%) |
|-------------------------|----|---------------------|--------|--------|----------|
|                         |    | 1 HEP               | 5 HEP  | 10 HEP |          |
| <i>D. pteronyssinus</i> |    | 1.12                | 1.59   | 1.58   | 6.5      |
| <i>D. microceras</i>    |    | 0.88                | 1.23   | 1.29   | 5.9      |
| Corr. Spearman          | rS | 0.85                | 0.75   | 0.69   | 0.96     |
|                         | P  | <0.001              | <0.001 | <0.001 | <0.001   |
| Diff. Wilcoxon          | P  | <0.01               | <0.01  | <0.01  | <0.01    |

\*Figures denote skin index (extract wheal area/histamine wheal area).

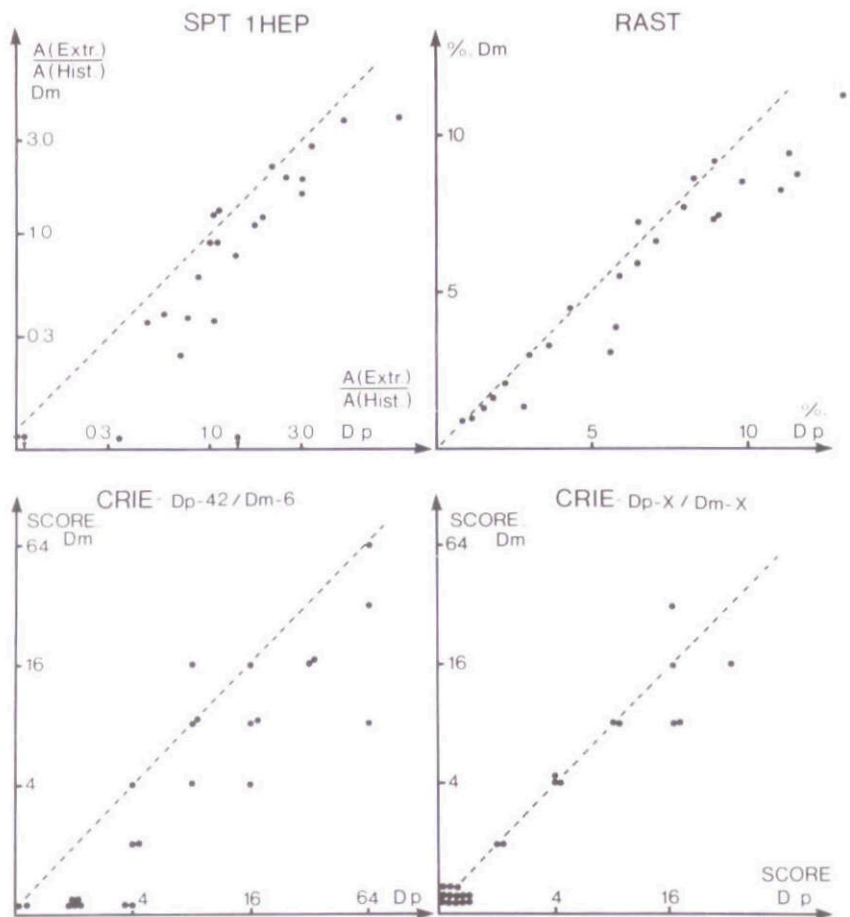


FIG. 2. Skin-prick test (skin index, 1 HEP), RAST (% retained activity), and CRIE responses to *D. pteronyssinus* (horizontal axes) and *D. microceras* (vertical axes). The broken lines indicate the 45° diagonal section through points of equal response to the two species.

Wilcoxon matched-pairs test. The same was true when RAST reactions to the two species were compared (Table I).

Weak correlations were obtained between the skin index (1 HEP) and RAST (Dp:  $r_s=0.27$ ;  $P>0.05$ ; Dm:  $r_s=0.38$ ;  $P<0.05$ ). The CRIE radiostaining scores obtained with sera eluted from the control columns, displayed a highly significant correlation between species (Dp-42 vs. Dm-6:  $r_s=0.92$ ;  $P<0.001$ ; Dp-X vs. Dm-X:  $r_s=0.99$ ;  $P<0.001$ ) (Fig. 2). The mean CRIE scores for Dp-42 were significantly larger than for Dm-6 when tested by the Wilcoxon test. No such difference was found for Dp-X vs. Dm-X.

The total CRIE scores for the two major

allergens correlated well with RAST (Dp:  $r_s=0.74$ ;  $P<0.001$ ; Dm:  $r_s=0.68$ ;  $P<0.01$ ).

Examples of CRIE autoradiographs after absorption are shown in Fig. 3. Absorption with heterologous species generally elicited a decrease in CRIE score compared with control column (Table II), indicating the presence of IgE antibodies with specificity common to both species. The homologous absorptions elicited maximum CRIE scores of 4 for Dp-42 and Dm-6 and 0 for Dp-X and Dm-X. Normal serum passed through the control column gave CRIE scores of 4 and 2 for Dp-42 and Dm-6, respectively, and 0 for Dp-X and Dm-X.

The maximum score for Dp-42/Dm-6

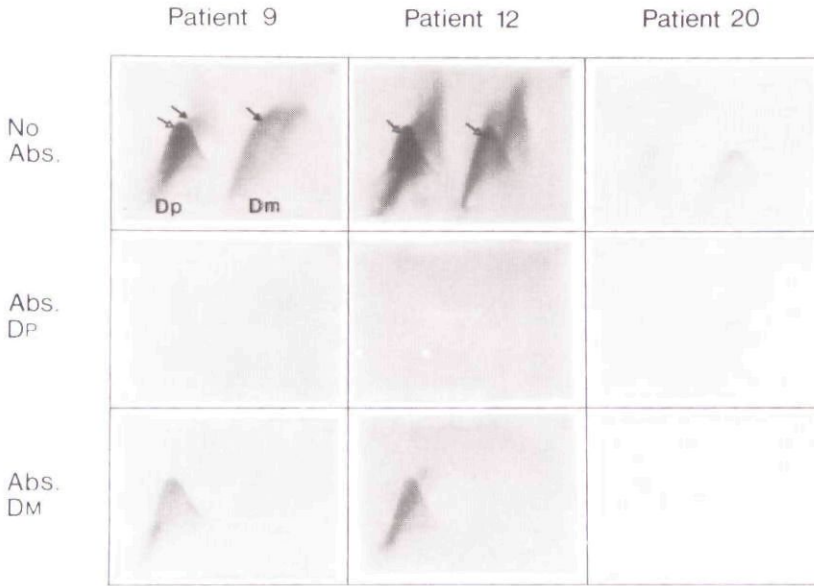


FIG. 3. Absorption CRIE autoradiographs for patients 9, 12 and 20. Horizontal row 1: serum passed through control column (Sephacrose 4B); row 2: serum absorbed on Dp-AML Sepharose; row 3: serum absorbed on Dm-AML Sepharose. Immunoplates: Dp-AML (15  $\mu$ g) and Dm-AML (15  $\mu$ g) electrophorized for 25 min in first dimension and overnight in the second dimension against rabbit anti-Dp-WMC (8.6  $\mu$ l/cm). After incubation with patient serum and radiolabelled anti-human IgE, autoradiography took place for 10 days at  $-20^{\circ}\text{C}$ . Open arrows point to precipitates Dp-42 and Dm-6, filled arrows to Dp-X and Dm-X.

obtained after heterologous absorption was 32 (Table II). For eight sera, a difference of at least two CRIE classes existed between heterologous and homologous absorption (in seven cases with specificity to Dp-42 and in one case for Dm-6).

A positive CRIE reaction to Dp-X after heterologous absorption was found in three patient sera (patient 4: score 2; patient 5: score 4, and patient 12: score 8). Dm-X bound no IgE after serum absorption with Dp-AML.

Examples of absorption SRID autoradiographs involving major allergens of three species: Dp-42, Dm-6 and Df-6 are shown in Fig. 4. Homologous absorptions reduced the radio-staining score to 0, with the exception of one case (patient 9: score Dp-42 (absDp)=1), where all scores for Dp were accordingly reduced one class.

Of 15 patient sera investigated, all but one of the unabsorbed sera displayed binding to each of the three applied allergens. All absorbed sera displayed one or more reactions with applied allergens. For nine patient sera with high IgE titres, the SRID autoradiographs were used to deduce the minimum number of antibody

specificities present. Only differences of at least two score classes between SRID reactions were taken into consideration in order to obtain a consistent interpretation of the pattern for each serum. Three levels of antibody specificity were considered: (1) reaction with epitopes (antibody-binding sites) common to the allergen of all three species (complete cross reaction), (2) reaction with epitopes common to two species, absent on the third, and (3) reaction with strictly species-specific epitopes present on the allergen of one species only. A schematic model for the classification of IgE-binding epitopes on the allergen molecule of each species is presented in Fig. 5.

The presence of antibodies with strict specificity for one species (Dp) was deduced in sera 5, 9, 12 and 14 (see Fig. 4), because these sera gave prominent reactions to applied Dp allergen after absorption with both Dm and Df. No combination of antibodies with restricted specificity could yield the same pattern for these sera.

Antibodies with specificity restricted to two species were demonstrated in serum 12, which

TABLE II. Dust allergen content by ELISA, RAST response and scores in absorption CRIE and SRID for 15 patients with high IgE levels

| Patient no. | Dust Ag (ng/g) |       |     |  | RAST (%) |      |       | Absorption CRIE*     |                     |                     |     | Antibody specificity§ by absorption SRID: |    |    |   |   |   |  |
|-------------|----------------|-------|-----|--|----------|------|-------|----------------------|---------------------|---------------------|-----|-------------------------------------------|----|----|---|---|---|--|
|             | Dp             | Dm    | Df  |  | Dp       | Dm   | Dp-Dm | Dp-42                | Dm-6                | Dp-X                | pmf | pm                                        | pf | mf | p | m | f |  |
| 1           | 1900           | 12000 | —   |  | 5.6      | 3.1  | +2.5  | 2 <sup>+</sup> (16)  | 1 (4)               | 0 (0)               |     |                                           |    |    |   |   |   |  |
| 4           | 125            | 250   | 15  |  | 11.6     | 8.7  | +2.9  | 16 <sup>±</sup> (64) | 1 (64)              | 2 <sup>±</sup> (16) | ±   | +                                         | ±  | —  | ± | — | — |  |
| 5           | 1400           | —     | —   |  | 9.1      | 7.4  | +2.7  | 16 <sup>±</sup> (32) | 0 (16)              | 4 <sup>±</sup> (16) | +   | —                                         | ±  | —  | + | — | — |  |
| 9           | 40             | —     | —   |  | 9.0      | 9.1  | -0.1  | 32 <sup>±</sup> (64) | 2 (8)               | 1 <sup>+</sup> (16) | +   | —                                         | +  | —  | + | — | — |  |
| 12          | 1400           | —     | —   |  | 9.9      | 8.5  | +1.4  | 16 <sup>±</sup> (64) | 1 (32)              | 8 <sup>±</sup> (32) | +   | +                                         | —  | —  | + | — | — |  |
| 14          | —              | —     | —   |  | 13.1     | 11.2 | +1.9  | 8 <sup>±</sup> (8)   | 4 (8)               | 0 (2)               | +   | —                                         | ±  | —  | + | — | — |  |
| 15          | 50             | —     | —   |  | 7.1      | 6.6  | +0.5  | 8 <sup>±</sup> (16)  | 1 (16)              | 0 (8)               | +   | ±                                         | ±  | —  | + | — | — |  |
| 16          | 50             | —     | —   |  | 5.9      | 5.5  | +0.4  | 8 <sup>±</sup> (16)  | 1 (8)               | 0 (8)               | ±   | —                                         | —  | —  | + | — | — |  |
| 17          | 300            | —     | —   |  | 9.0      | 7.3  | +1.7  | 8 (32)               | 1 (16)              | 0 (16)              | ±   | ±                                         | —  | —  | + | — | — |  |
| 19          | 40             | —     | —   |  | 5.8      | 3.9  | +1.9  | 4 (4)                | 2 (2)               | 0 (0)               | —   | —                                         | —  | —  | — | — | — |  |
| 20          | 7800           | 7500  | 15  |  | 6.5      | 7.2  | -0.7  | 4 (8)                | 8 <sup>±</sup> (16) | 0 (0)               | ±   | ±                                         | —  | +  | — | ± | — |  |
| 21          | 300            | —     | 540 |  | 8.3      | 8.6  | -0.3  | 4 (4)                | 4 <sup>±</sup> (4)  | 0 (0)               | ±   | —                                         | —  | —  | — | — | — |  |
| 22          | 380            | —     | —   |  | 8.0      | 7.7  | +0.3  | 4 (8)                | 4 (8)               | 0 (2)               | —   | —                                         | —  | —  | — | — | — |  |
| 24          | 4400           | —     | —   |  | 11.4     | 9.4  | +2.0  | 4 <sup>±</sup> (8)   | 2 (4)               | 0 (0)               | —   | —                                         | —  | —  | — | — | — |  |
| 25          | 3800           | —     | —   |  | 11.1     | 8.2  | +2.9  | 4 <sup>±</sup> (16)  | 2 (8)               | 0 (4)               | +   | —                                         | ±  | —  | + | — | — |  |

\*Figures denote scores after heterologous absorption. Scores after passage of control column shown in brackets.

†1 CRIE class above homologous absorption.

‡>1 CRIE class above homologous absorption.

§Presence of antibodies to epitopes with the denoted species specificity; —: absence of antibody binding; ±: presence of antibody binding possible. The letter code for epitopes is explained in Fig. 5.

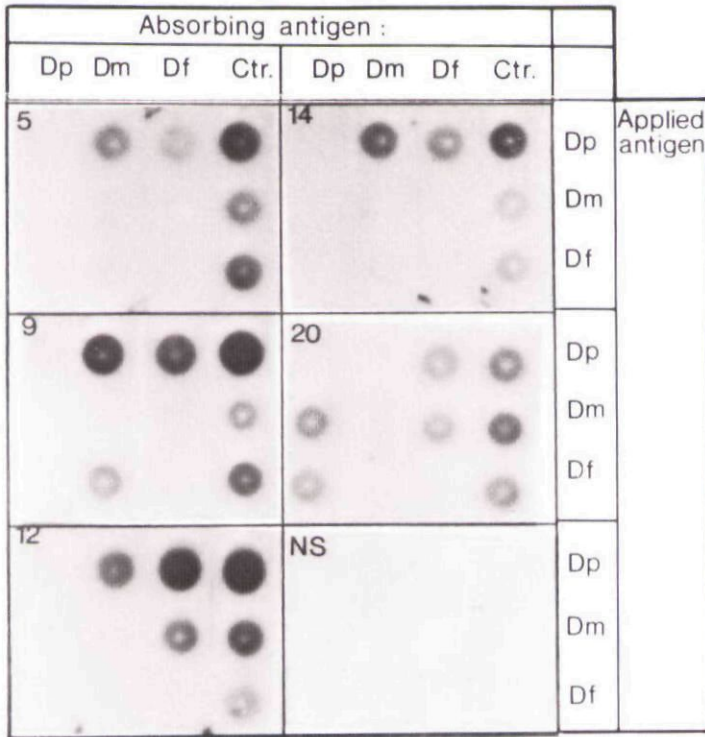


Fig. 4. Absorption SRID autoradiographs of five patient sera (nos 5, 9, 12, 14 and 20) and normal serum (NS). Antigens applied in SRID were purified major allergens Dp-42 (0.2  $\mu$ g), Dm-6 (0.16  $\mu$ g), and Df-6 (0.4  $\mu$ g). Diffusion took place for 48 h against mixed monospecific rabbit antibodies to Dp-42, Dm-6 and Df-6. Separate gel strips, each with three diffusion precipitates, were incubated with serum passed through the following columns: (1) Sepharose 4B (Control), (2) Dp-AML Sepharose 4B, (3) Dm-AML Sepharose 4B, and (4) Df-AML Sepharose 4B. After incubation with radio-labelled anti-human IgE, autoradiography took place for 7 days at  $-80^{\circ}\text{C}$ .

displayed IgE-binding to Dm after absorption with Df, but not after absorption with Dp. Allergen Dm-6 was thus assumed to contain at least one antibody-binding site (epitope) shared with Dp-42, but absent on Df-6. Analogously, serum 9 contained IgE antibodies binding to a Df-6 epitope shared with Dp-42, but absent on Dm-6. Serum 20 contained an epitope shared between Dm and Df, absent on Dp.

Sera that reacted to applied antigens only when unabsorbed, were assumed to contain antibodies to common epitopes. Sera 5, 9 and 14 in Fig. 4 reacted with Dm in the unabsorbed state only, and this reaction thus represented antibodies of common reactivity. An analogous reaction to Df was observed in the case of serum 12.

Table II presents a summary of the

specificities whose presence was deduced in the nine sera investigated that had the highest IgE titres. Reactions to common epitopes and to strictly species-specific epitopes on Dp were most frequent (6 and 7 sera, respectively), but IgE antibody with specificity restricted to two species was present, representing all three possible two-species combinations. Thus sera 4 and 12 reacted to an epitope shared by Dp and Dm, serum 5 to an epitope shared by Dp and Df, and serum 20 to an epitope shared by Dm and Df.

## DISCUSSION

A study was made of the species specificity of antibody responses to allergens of *Der-*

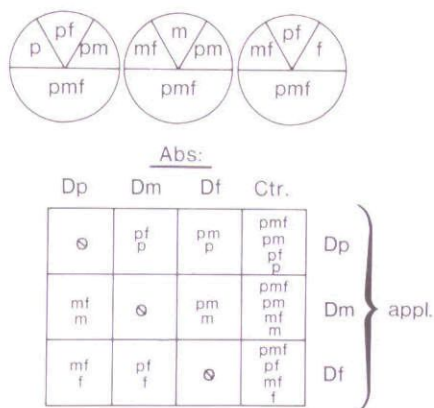


FIG. 5. A model for the classification of IgE-binding epitopes on allergens Dp-42, Dm-6 and Df-6. Single letters denote strictly species-specific epitopes; p: on Dp; m: on Dm; f: on Df. Two-letter combinations denote epitopes common to two species, unshared by the third; pm: common to Dp and Dm; pf: common to Dp and Df; mf: common to Dm and Df. Epitopes common to all three species are denoted pmf. At the bottom of each matrix cell are listed the possible IgE specificities associated with a positive radiostaining at the corresponding position on the absorption SRID autoradiographs (see Fig. 4).

*matophagoides* dust mites in a Belgian patient population.

Highly significant correlations between patients' reactions to standardized *D. pteronyssinus* and *D. microceras* extracts in skin-prick test and RAST were found (Table I). This is a common observation when diagnostic reactions to house dust mites belonging to the genus *Dermatophagoides* are compared in population studies [17, 20]. In the present patient population, however, marginally larger (but statistically highly significant) reactions to Dp in both SPT and RAST were found (Table I). This could not be explained by different allergenic potencies of the test extracts, which were standardized and adjusted to contain approximately equal weights of major allergens, Dp-42 and Dm-6 [22].

CRIE reactions to Dp-42 were significantly larger than to Dm-6, a difference not observed between the other corresponding major allergens, Dp-X and Dm-X. On the basis of these data, the existence of species-specific IgE antibodies to Dp-42 had to be assumed, and the presence of such antibodies was readily

demonstrated in both absorption CRIE and absorption SRID (Table II). Although species-specific IgE were also present against Dp-X, reactions were fewer and less intense.

The observed occurrence of Dp as the dominating species in this area (Fig. 1) is in good agreement with previously published housedust mite investigations from the same geographical region [1, 6, 19]. The dust sampling in the present study took place in spring and early summer, when indoor humidity conditions are unfavourable to mites, a fact which serves to explain the rather low combined levels of mite allergen/gram dust (maximum 15,000 ng/g) with maximum levels of 300,000 ng/g reported from UK [21] and Denmark [11].

The predominant sensitization to allergens of Dp in this area thus seems to be the explanation for the recorded larger mean response to Dp in diagnostic tests and the frequent occurrence of serum IgE antibodies strictly specific for Dp-42. The presence in most sera of IgE antibodies responding to common epitopes on Dp-42/Dm-6/Df-6 and on other allergens such as Dp-X and analogous allergens serves to explain the concomitant high levels of correlation (cross-reaction) between species obtained in the diagnostic tests.

These findings stress the importance of selecting allergen preparations from the proper species (or species mixture) for diagnosis and immunotherapy of allergic diseases based on prevalence data for the given geographical area. Correct choice of species is necessary for optimization of clinical efficacy, although at present it is not known whether the effect is important or only marginal in immunotherapy.

A good agreement existed between results of absorption CRIE and absorption SRID. Sera with strong CRIE reaction to Dp after absorption with Dm generally contained IgE antibodies to epitopes specific to Dp, as deduced from absorption SRID (Table II). Serum 20, from a patient exposed to high levels of Dm, reacted strongly in CRIE to Dm-6 after absorption with Dp, and contained antibodies to an epitope on Dm-6 that was shared by Df-6, as deduced from absorption SRID. Besides confirming the presence of species-specific IgE antibodies to Dp-42, the incorporation of all three species in the SRID absorption in many cases allowed the distinction between strict species specificity and specificity to epitopes shared by two species.

Species-specific antibody responses to house dust mite major allergens have been demonstrated recently in laboratory animals like rabbits [8, 10] and mice [4, 5]. Polyclonal rabbit antisera raised against Dp-42, Dm-6 and Df-6 elicited strong responses to strictly species-specific epitopes on Dp, Dm and Df, respectively. One rabbit further produced antibodies to epitopes shared by Dm-6 and Df-6 [10]. Species-specific reagents could be developed and employed in an ELISA for independent measurement of the three *Dermatophagoides* species [11].

Mouse hybridoma antibodies raised against P1 (=our Dp-42 [9]), the majority of which were restricted to two clonal specificities, did not bind to the corresponding Df allergen, and were considered species-specific [4]. Human sera from patients allergic to mites elicited up to 85% inhibition of the antigen binding to the hybridoma antibodies [4]. Although overlapping or close topographical proximity between common and specific epitopes [2] may have been partially responsible for the inhibitory effect, we assume that part of the human IgE or IgG antibody specificity detected by Chapman *et al.* [4] corresponded to epitopes specific to Dp-42 or shared by Dp-42 and Dm-6, as defined in our model.

The postulated model for classification of antibody specificity (Fig. 5) was fully compatible with previously published findings from rabbit antibodies and with the IgE absorption data obtained in the present study. This was not the case with alternative models involving less than four classes of epitopes on each species antigen. The model provides a suitable framework for further characterization of the immunologically functional sites on the surface of this group of major dust mite allergens and for studies of the dynamics of antibody production and modulation during e.g. immunotherapy. Mapping of hybridoma antibody specificity on the basis of this model is in progress in our laboratory [7]. It has, however, yet to be established to what degree epitopes of the various classes are topographically distinct or overlapping and to what extent immunodominant epitopes are of a sequential or a conformation-dependent nature. Amino acid sequence data are useful in this context, and such analyses are now being undertaken on major allergens of the three species.

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