

Standardization of Mite Extracts

Qualitative and Quantitative Investigations of Three Kinds of Preparations with CRIE and RAST

PETER LIND

The Protein Laboratory, University of Copenhagen, Copenhagen, Denmark

Allergen extracts of the house dust mites (fam.: Pyroglyphidae) may be produced in two principally different ways: 1) The full-grown, whole mite culture, containing the mites and their excreta, may be used directly as the starting material for extraction, as described in (6). 2) Alternatively, the mite bodies themselves may be separated from the substrate by various techniques and extractions performed on these. Both kinds of extracts are used at present in clinical practice.

Radioimmunochemical techniques, supplemented with skin-prick-test, have previously been applied to standardization and batch-control of commercial *D. pteronyssinus* mite extracts (2). In this case, extracts of very closely related antigenic composition were compared, and good agreement between results of three standardization methods employed was found.

The present study was undertaken to investigate the composition and allergenic potency of mite extracts produced on the basis of different starting materials, employing two techniques currently in use for allergen standardization: CRIE and RAST.

MATERIALS AND METHODS

D. pteronyssinus mites were grown on a mixture of human dander from electric shavers and a dried yeast product. From the same full-grown mite culture, three fractions were produced:

1) A mite body fraction, obtained after sieving on 212 μm and repeated flotation on saturated NaCl-solution. Control experiments assured no loss of allergenic material during the flotation step. 2) An excreta-fraction was collected after sieving on a 44 μm sieve. 3) The third fraction consisted of a sample of the original dried, whole culture.

Microscopical examination revealed the mite body fraction to consist primarily of intact mite bodies and to a smaller extent of mite fragments. Contamination with mite fecal pellets and culture medium particles was less than 2% on a volume basis. The excreta fraction was free from visible mite fragments and consisted of a mixture of fecal pellets and culture medium particles. The yield of dried mite bodies was 8%, relative to dried, whole culture. From the three fractions and the two culture medium components extracts were produced, following the procedure in (5). The extracts were dialyzed, freeze-

dried and reconstituted in 50% glycerol-saline.

The rabbit antibodies employed originated from the DAS-76 study (4), and were produced by immunization with a whole culture extract. Three pools of serum from mite-allergic patients were used in CRIE and/or RAST: 1) Pool 1 was a mixture of 11 sera from the DAS-76 study. 2) Pool 2 was a gift from Diagnoselaboratoriet, Copenhagen, containing sera from 29 patients with more than 50 sorbent-units in the AI ((OH)₃-RAST. 3) Pool 3 was a mixture of serum from British patients (NIBSC lit no. 584, kindly provided by Dr. W. D. Brighton, NIBSC, London). Control serum for RAST and CRIE was from a timothy-allergic patient.

Radiolabelled antiserum to human IgE was bought from Pharmacia A/B, Uppsala.

Crossed immunoelectrophoresis (CIE) and Crossed Radio-Immunoelectrophoresis (CRIE) were performed as described in (3). Activated paper-discs for use in RAST and RAST-inhibition were produced according to (1).

Absorption experiments to control the specificity of the rabbit antibodies were carried out according to (4). In short, immobilized rabbit antibodies to *D. pteronyssinus* and *Avena elatior* pollen (the latter as control) were produced by reaction with CNBr-activated Sepharose 4B (Pharmacia) and each packed on a column. Sixty microgrammes mite-body extract were applied to the columns and eluted with citrate-buffer, pH 6. In each, 12 fractions were collected which were reacted with three activated paper-discs. These were used as allergosorbents in RAST with serum pool 1.

RESULTS

RAST discs produced from fractions passing through the columns with immobilized anti-*Avena* antibodies gave counts in the RAST ranging from 3.5 to 31% of the radioactivity added, whereas the disc made from extracts, absorbed with anti-mite antibody showed corresponding figures in the range 1.5–5%. Estimated from the summed RAST counts in all fractions, the rabbit antibodies against whole culture extract absorbed 84% of the allergenic activity in the mite body extract.

The precipitation pattern visualized after protein-staining of CIE-plates with the three mite fractions is illustrated in Fig. 1. A total of 20, 26 and 27 precipitates could be counted on plates with mite body, whole

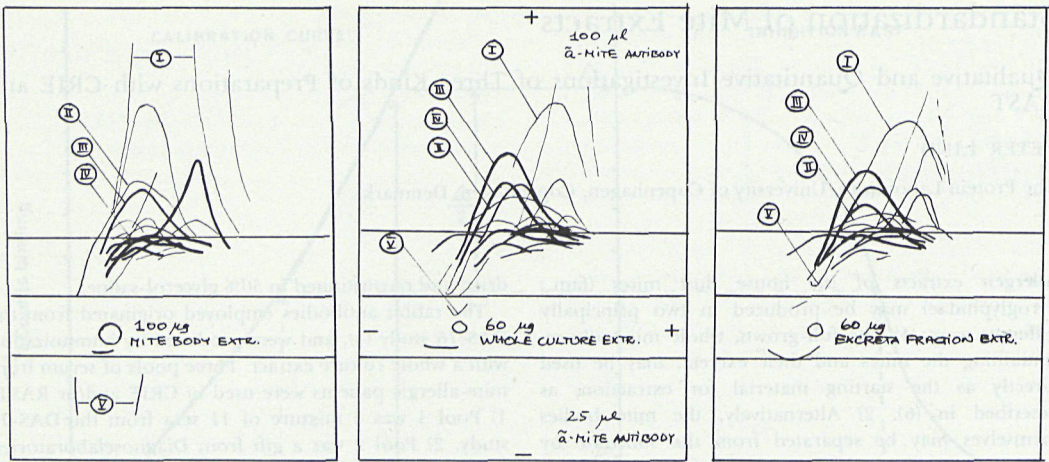


Fig. 1. Drawing of Crossed Immunoelectrophoretic pattern obtained with three different mite extracts. A) 100 µg mite body extract, B) 60 µg whole culture extract, and C) 60 µg excreta fraction. Allergens, as identified in CRIE, indicated by numbers I–V.

TABLE 1
Composition and relative allergenic potency of three mite extracts, determined by CRIE, RAST and RAST-inhibition

	Mite body extract	Whole culture extract	Excreta fraction extract
<i>CRIE</i>			
(a) Amounts of individual major allergens/mg, relative to whole culture extract			
I	200	100	90
II	100	100	70
III	60	100	80
IV	15	100	60
V	300	100	< 100
<i>Individual RAST's</i>			
(b) Mean RAST-counts (%)			
(71 patients)	18.3	16.8	16.6
Slope of regression-line with RAST-counts plotted against whole culture	0.98 ^a	1.00	0.97
<i>RAST-inhibition</i>			
(c) Potency in RAST-inhibition, relative to whole culture extract, using discs with extracts from:			
Mite-body	200	100	40
Whole culture	40	100	220
Excreta	80	100	160
Pharmacia	50	100	50

^a Two patients excluded.

culture and excreta fraction extract, respectively. Common antigens in the three fractions were identified by tandem-CIE and additional experiments. Quantitation of individual antigen amounts took place on 5×5 cm plates without intermediary gel. The precipitation pattern was both qualitatively and quantitatively very similar when extracts of whole culture and extract of excreta were compared. This reflects the fact that the full-grown mite culture consists primarily of excrements and undigested medium particles, with only 5–8% mite bodies. On the other hand, the precipitation pattern of the mite body extract was quantitatively very different being present in more than 10 times larger amount in the mite bodies than in the whole culture extract and vice versa. Several intensely stained precipitates represented human dander proteins in the whole culture and excreta fraction extracts. The yeast component showed no reaction with the rabbit antibodies.

CRIE-experiments were carried out on all these fractions. Control plates incubated with serum from a pollen-allergic patient were negative throughout. Optimal amount of application was established for the mite body extract and found to be 100 μ g. Five IgE-binding precipitates were revealed in all three fractions, using pool 1. The corresponding allergens were numbered I–V (Fig. 1). The intensity of radiostaining was most prominent with allergens I, II and IV. Serum pools 2 and 3 gave essentially the same result, with specific radiostaining of allergens I, II, III, IV and V, although the relative staining intensity varied between the identified allergens, depending on which serum pool was used. None of the precipitates representing human dander components showed specific radiostaining.

Using a whole culture extract and the same rabbit antibodies as in this study, 1 major allergen and several minor allergens from *D. pteronyssinus* were identified in the DAS-76 standardization project (4), using serum from 31 individual patients. By cross-identification, allergen IV was found to be identical with the major allergen, while allergens I, II and III corresponded to minor allergens in the DAS-76 study.

Quantitation of the individual allergens showed allergens I and V to be present in the largest amount in the mite body extract, whereas allergen IV was found to be associated with the whole culture and excreta fraction (Table Ia).

RAST was performed on 71 individual patient-sera (from the DAS-76-study) using discs coupled with extracts of all three mite fractions in the same concentration. Results are summarized in Table Ib. A close correlation was found between RAST counts on discs prepared from whole culture and excreta fraction extract, ($r = 0.99$). Statistical analysis on the RAST results obtained using discs with mite body extract showed a significantly better correlation with results from whole culture discs if two patients with 3–4 times higher RAST counts on mite body discs were omitted from the regression analysis. The RAST counts for the

remaining 69 patients showed a close correlation between the IgE-binding capacity of discs produced from whole culture extract and discs produced from mite body extract ($r = 0.98$). Judged from the mean RAST counts and the slopes of the regression lines (Table Ib) the mite discs had the same IgE-binding capacity. RAST on discs prepared from human dander and yeast extracts gave mean counts of 1.4% and 1.3%, respectively. Contribution of possible allergens in the culture medium to the IgE-binding capacity of the mite discs was considered negligible.

RAST-inhibition experiments were performed with discs prepared from all three fractions and discs bought from Pharmacia A/B. All three fractions were used as inhibitors towards serum pool 1. Altogether 12 inhibition-curves were plotted, and allergenic potency, relative to whole culture extracts was computed (Table 1c). The relative potency of the mite body extract varied from 40 to 200%, the highest relative potency being obtained when mite body discs were used as allergosorbent. The same tendency was observed when the excreta fraction extract was used as inhibitor, the highest potency being determined with discs prepared from extract of the same (or closely related) composition. Judged from the RAST-inhibition experiments, all three mite fractions were of fairly equal potency.

DISCUSSION AND CONCLUSION

In spite of large quantitative differences in the content of individual allergens, as revealed by CRIE, the three mite preparations studied were of fairly equal potency, when analyzed in the RAST system. This may be explained by the fact that some allergens predominantly appeared in the mite body extract, others in the whole culture and excreta fraction extract.

If relative potency had been estimated solely by quantitation of the major allergen (no. IV) identified in CRIE (4), there would have been gross disagreement with the results of RAST-inhibition. When the other allergens identified in CRIE with the serum pools are taken into consideration, e.g. by calculation of the geometric mean of the relative allergen amounts, this estimate of relative allergenic potency would be in better agreement with the RAST results.

The present study may exemplify the situation when reference materials for use in standardization are to be selected. In cases where the choice of starting material for extraction is not consented upon beforehand — as in the case of house dust mites — detailed studies will be necessary to ensure representation of all important allergenic components in the reference material.

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Address:

Peter Lind

University of Copenhagen

Protein Laboratory

Sigurdsgade 34

DK 2200 Copenhagen N

Denmark

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