GEL-FILTRATION ANALYSIS OF PROTEIN ANTIGENS OF THE MITE Dermatophagoides pteronyssinus

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Data published in the last decades [1, 3-5, 7, 10, 11] show that mites of house dust, especially those belonging to the genus Dermatophagoides, possess allergenic properties and may perhaps play an essential role as allergenic factor in the development of atopic (allergic) diseases in man. Successful specific hyposensitizing therapy with homologous allergen has also been reported in patients with atopic bronchial asthma with increased sensitivity to house-dust mites [1, 6, 8, 12]. Our previous investigations suggest that mites of the genus Dermatophagoides possess a sufficiently complex range of allergenic components. The study of the structure (composition) of the mite allergen and of the allergenic and immunogenic properties of its various fractions would enable methods of obtaining allergenic preparations with assigned allergenic and immunogenic characteristics to be obtained for the purpose of specific diagnosis and treatment.

This paper gives the results of a study of the structure of an extract of a culture of the mite D. pteronyssinus by gel-filtration on Sephadex followed by determination of the allergenic and immunochemical activity of its various chromatographic fractions.

EXPERIMENTAL METHOD

A 6% saline extract of a culture of the mite D. pteronyssinus was used for gel-filtration. According to data in the literature [4, 11], mites of this species are the most widespread house-dust mites with allergenic activity. Gel-filtration was carried out on Sephadex G-25 and G-75 (Pharmacia, Sweden). One-stage gel-filtration was carried out on a column measuring 1.6 × 60 cm at the rate of 9-10 ml/h. Mite extract was applied to the column with gel in a volume of 3 ml (1.2-1.5 mg of protein nitrogen). Elution was carried out with 0.2 M phosphate buffer with 0.2 M NaCl at 20°C. Samples, 3 ml in each tube, were taken by an automatic collector. The protein content in the fractions studied was determined on an SF-4A spectrophotometer at a wavelength of 280 nm and the protein nitrogen concentration was measured by the micro-Kjeldahl method. The free volume of the column was determined with the aid of blue dextran. The allergenic activity of the fraction was investigated by the direct intradermal test on guinea pigs sensitized by five subcutaneous injections of the 6% extract of a culture of D. pteronyssinus. Immunochemical activity was studied in Ouchterlony's precipitation tests with specific rabbit antisera.

To determine the molecular weight of the proteins of the mite extract, protein substrates of known molecular weight were used: ribonuclease (13,600), egg albumin (45,500), and bovine serum albumin (67,000). The molecular weight also was calculated by the empirical formula for Sephadex G-75: $M = 5.624 - 0.752 \times (V_e/V_0)$, where V_e is the effluent volume and V_0 the external (free) volume of the particular column used [2].

EXPERIMENTAL RESULTS

During fractionation of the extract of the culture of D. pteronyssinus on a Sephadex G-25 column, all the allergen left the column in the free volume. The range of protein fractionation on Sephadex G-25 extends up to a molecular weight of 5000 [1].

Gel-filtration on Sephadex G-75 enabled the characteristic dependence of allergenic and immunochemical activity of the various elution fractions on protein content and molecular weight to be established. We know [2] that protein structures with a molecular weight of over 75,000 are eluted from Sephadex G-75 in the free volume. The free volume of the Sephadex G-75 column was 46 ml and of Sephadex G-100 column 41 ml.

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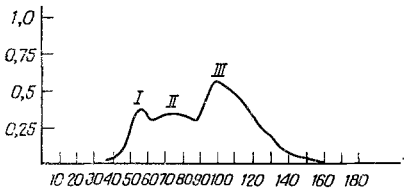

Fig. 1. Fractionation of extract of culture of mite D. pteronyssinus by filtration through Sephadex G-75. Abscissa, volume of eluate (in ml); ordinate, optical density at 280 nm. I, II, and III) Protein fractions of mite extract.

TABLE 1. Results of Direct Intradermal Test with Various Elution Fractions of Extract of D. pteronyssinus Culture on Sensitized Guinea Pigs (M ± m; n = 6)

Allergen	Mean dilution of allergen giving positive reaction
Whole extract of D. pteronyssinus culture	1:554±103,15 (100%)
Fraction I	1:320±64,09 (68%)
Fraction II	1:299±42,82 (54%)
Fractions I+II	1:938±85,6 (169%)
Fraction III	1:2±0,51 (0,4%)

Legend. Comparative allergenic activity of mite extract shown in parentheses.

It will be clear from Fig. 1 that the D. pteronyssinus extract separated into three fractions during gel-filtration on Sephadex G-75. Fraction I, with an elution volume of about 21 ml and molecular weight of about 49,200, was immunochemically and allergologically the most active. On immunochemical analysis with rabbit antiserum this fraction formed one precipitation line. Whereas the 6% (0.4-0.5 mg protein nitrogen/ml) whole extract of D. pteronyssinus caused a positive skin reaction in sensitized guinea pigs in dilutions of between 1:256 and 1:1024 (1:554 ± 103.15), the allergen leaving the column in fraction I of the eluant, containing the same protein nitrogen concentration, caused a similar reaction in dilutions of 1:128-1:512 (1:320 ± 57.7), so that its activity was about 58% of that of the 6% mite allergen. Fraction II, with an elution volume of about 30 ml, and with a protein concentration a little lower than in fraction I (Fig. 1), was immunochemically inert, but its allergenic activity was sufficiently high, namely 1:256-1:512 (1:299 ± 42.8), or about 54% of that of the 6% mite culture extract. The molecular weight of this fraction was about 28,000. Since the allergenic activity of fractions I and II of the eluant separately, with the same protein nitrogen concentration, was lower than that of the whole 6% extract, the allergenic potency of fractions I and II taken together also was investigated. It was found that whereas the whole 6% mite extract caused a positive skin reaction in sensitized guinea pigs in a mean dilution of 1:554, the mixture of antigens eluted on gel-filtration in fractions I and II gave a similar reaction in a dilution of 1:939 ± 85.6, equivalent to 169% of the allergenic activity of the 6% extract (Table 1). During immunochemical analysis the mixture of these fractions formed as a rule one or two precipitation lines. Fraction III, with a small volume (about 54 ml) and with the highest protein concentration (Fig. 1), on the other hand, possessed extremely low allergenic activity (1:2 ± 0.51), and it was immunochemically inert. Its allergenic activity in the direct skin test was only about 0.4% of the total allergenic activity of the 6% mite extract, despite a higher concentration of proteins with a molecular weight of about 12,000 or below.

Consequently, the immunogenic and allergenic activity of the D. pteronyssinus extract was probably due to protein components of its elution fractions I and II with molecular weights of about 28,000-49,000. Elution fraction III with a molecular weight of under 12,000 was immunochemically inert and had little allergologic activity, which suggests that pigment proteins with low antigenic activity were contained in this fraction.

Unlike us, Miyamoto et al. [9] carried out gel-filtration of an extract of a culture of the mite Dermatophagoides farinae on Sephadexes G-50 and G-2000 and they showed that the molecular weight of the strongest antigen of house dust and of the mite D. farinae must be above 10,000 but below 69,000 and, in all probability,

between 40,000 and 50,000. In their opinion the allergenic activity of the mite extract does not correspond to a peak of optical density at 280 nm.

Fractionation and investigation of the structure and properties of the separate elution fractions of the multicomponent antigenic complex of the mite D. pteronyssinus thus demonstrated the molecular heterogeneity (although with a narrow spectrum of molecular weights) of the immunochemically and allergologically most active components of an extract of a culture of D. pteronyssinus and also showed that, in principle, it is possible to separate and prepare allergens with assigned parameters required for specific diagnosis and treatment.

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