

Antigens Common to Scabies and House Dust Mites

EDVARD S. FALK, SIRI DALE, ROALD BOLLE and BJØRN HANEBERG

Dept. of Dermatology and Dept. of Pediatrics, University of Tromsø, Tromsø, and Nyco & Co. A/S, Oslo, Norway

Twenty-five antigens were demonstrated in a crude *Dermatophagoides farinae* (DF) extract by means of crossed immunoelectrophoresis (CIE), using a rabbit anti-DF serum. With the same antiserum, four antigens were demonstrated in a scabies mite preparation, indicating an immunological relationship between these scabies antigens and some of the DF antigens. In tandem CIE experiments the DF antigens were found to be partly identical to three of the scabies antigens. No IgE activity against any of these scabies antigens was evident in pooled sera from patients with house dust mite allergy by crossed radioimmuno-electrophoresis. However, one such patient had IgE antibodies to a DF antigen and a partial identical scabies antigen.

Key words: *Dermatophagoides farinae* (DF) antigens; scabies mite antigens.

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Scabies infestation involves the immune system, and specific hypersensitivity against the scabies mite has been demonstrated by *in vivo* skin tests (4). A large proportion of patients with scabies infestation have increased serum concentrations of IgE (2). By means of radioallergosorbent test (RAST), the specificity of the IgE antibodies was shown to be partly directed against house dust mites (3). Many of the patients had no disposition for or history of atopy. Decreased concentrations of both total and specific serum IgE some months after treatment for the scabies infestation, indicate that the scabies mite had been the stimulus for the IgE production (5).

In the present report the relationship between the antigens of the scabies mite and the house dust mite *Dermatophagoides farinae* (DF) was investigated by means of immunological methods. DF was used because this mite is easier to cultivate than *D. pteronyssinus* (DP) and was therefore first produced by Allergon AB. However, different *Dermatophagoides* species cross-react, as shown for DP and DF (3).

MATERIAL AND METHODS

Scabies mite preparation

Eight hundred scabies mites (3 mg) were collected from a patient with scabies crustosa by removing the mites undamaged from the patient to a clean glass cell with a cork. They were stored at a temperature of -20°C , crushed in a mortar with a glass rod and mixed with 100 μl 0.125 M ammonium bicarbonate (NH_4HCO_3) pH 8.0 and 2% *n*-butanol. The suspension was stored at -20°C before being tested by immunoelectrophoresis.

Crude extracts of Dermatophagoides farinae (DF) mites

Disrupted, freeze-dried DF house dust mites (Batch No. 087795, Allergon AB, Engelholm, Sweden) were extracted in a 4% (w/v) solution with 0.125 M ammonium bicarbonate pH 8.0 and 2% (v/v) *n*-butanol. The suspension was stirred for 20 h at 6°C (Rosenthal/325-1, 200

rpm) and centrifuged at 27,300 *g* for 60 min (Sorvall, Superspeed RC2-B). The sediment was resuspended in half of the initial volume of 0.125 M ammonium bicarbonate pH 8.0 and 2% *n*-butanol, and the suspension was extracted and re-centrifuged. The two supernatants were filtrated (Acropore 0.8 μ m, Gelman), pooled and freeze-dried. A freshly prepared solution (9 g of dry weight/l) of the freeze-dried material was used for immunoelectrophoresis.

The extraction of the mites for immunization in rabbits was performed as described above, with the exception of using 0.075 M physiologically buffered saline (pH 7.2) containing 2% (v/v) *n*-butanol, instead of ammonium bicarbonate buffer. The two pooled supernatants were concentrated, using Diaflo UM 2, Amicon (cut off $M_w \sim 1000$) membrane in an Amicon cell.

Human epithelium preparation

5 mg human dander was mixed with 200 μ l 0.125 M NH_4HCO_3 pH 8.0 and 2% *n*-butanol, and crushed in a mortar before use.

Human sera

Sera from 36 patients with house dust mite allergy and IgE antibodies to the house dust mites DP or DF were pooled. Serum from one patient ($\emptyset$.B.) with high levels (RAST class 4) of IgE antibodies to the mite DF was also obtained.

Rabbit antisera

Rabbit antisera to DF were prepared as described by Harboe & Ingild (6). Three rabbits were immunized and boosted every month and bled 14 days after each immunization. Pooled antisera, obtained 10 to 14 months after the first immunizations, were used in these experiments and denoted anti-DF.

Crossed immunoelectrophoresis (CIE)

CIE was performed on glass slides (5 $\times$ 7 cm) essentially as described by Weeke (10) using a 1% agarose gel (Litex, Glostrup, Denmark)) containing a buffer of 0.073 M Tris, 0.024 M

barbital, 0.006 M calcium lactate and 0.003 M sodium azide (pH 8.6, 22°C). The first and second dimension electrophoreses were run at 12°C at 10 V $\times$ cm⁻¹ for 40 min and at 2 V $\times$ cm⁻¹ for 19 h, respectively. Two patterns of gel sections were used.

Pattern A: 5 $\times$ 4.5 $\times$ 0.11, 5 $\times$ 1.5 $\times$ 0.13 and 5 $\times$ 1 $\times$ 0.15 cm, respectively, denoted from the anode. 500 μ l anti-DF was added to the gel section 1 from the anode.

Pattern B: 5 $\times$ 3.5 $\times$ 0.10, 5 $\times$ 1.5 $\times$ 0.11, 5 $\times$ 1 $\times$ 0.13 and 5 $\times$ 1 $\times$ 0.15 cm, respectively, denoted from the anode. 400 μ l of anti-DF were added to the gel section 1 and 100 μ l to the gel section 4 from the anode.

The slides were pressed, washed, dried and stained with Coomassie brilliant blue R[®]. Heating of slides to be used in CRIE was avoided. Such slides were stored dry at 5°C until use and were not stained with Coomassie brilliant blue R until after completion of the autoradiography.

Crossed radioimmuno-electrophoresis (CRIE)

CRIE was performed essentially as described by Løwenstein et al. (8). The CIE preparations were incubated for 24 h at 25°C with 0.4 ml of either the pooled human sera or patient $\emptyset$.B. serum in the incubation buffer (0.025 M phosphate buffer pH 7.5, containing 0.3% (w/v) of sodium azide, 2% (w/v) Tween 20 and 0.1% (w/v) sodium chloride). After washing four times for 10 min with 7 ml 0.9% (w/v) sodium chloride, the slides were incubated with ¹²⁵I-anti-IgE (Phadebas[®] RAST isotope) solution, approximately 250,000 ct/min (LKB Wallace) in 7 ml of incubation buffer for 24 h at 25°C. After washing the slides four times for 10 min with 7 ml of 0.9% (w/v) of sodium chloride and once with distilled water for 10 min, they were dried and laid face down on X-Omat (Kodak) X-ray films for autoradiography. A paper disc reference system in CRIE was used as described by Aukrust et al. (1). The autoradiography was carried out until radiostaining could be obser-

ved from precipitates giving CRIE-class F according to the grading system.

Tandem crossed immunoelectrophoresis

Tandem CIE was performed as described by Krøll (7). Various volumes of the crude mite extract and the fraction to be investigated were applied in two holes 1 cm apart for the first dimension electrophoresis (Figs. 3, 4 and 5). Further experimental details were identical to the standard CIE preparation, using pattern A.

RESULTS

Twenty-five antigens were demonstrated in a crude DF extract by means of CIE-techniques, using the anti-DF serum (Fig. 1a). Using the same antiserum, four antigens were demonstrated in the scabies mite preparation, indicating an immunological relationship between these scabies antigens and some of the DF antigens (Fig. 2a).

Two tandem CIE-experiments were performed to identify the DF-antigens which were related to the scabies antigens (Figs. 3 and 4a). In tandem CIE-experiments, a DF-antigen seemed to be partly identical with the scabies antigens

No. 1 and No. 2 (Fig. 3a), while another DF-antigen was partly identical with the scabies antigen No. 3 (Fig. 4a). No DF-antigen, related to the scabies antigen No. 4, could be identified in these experiments.

The scabies mites live on human epithelium which is also a common cultivation medium for the house dust mites. A tandem CIE-experiment was therefore performed with a human epithelium extract which did not give rise to any immunoprecipitates. This demonstrates that none of the antigens which were identified with the anti-DF serum, originated from human epithelium (Fig. 5a).

After this, two CRIE-experiments were performed using one patient serum pool and one individual patient serum (the Ø.B. serum), to identify possible IgE antibodies to scabies antigens. This showed that the patient serum pool reacted to four DF antigens (Fig. 1b). None of the scabies-immunoprecipitates gave rise to radiostaining using the serum pool (Fig. 2b) and therefore none of these antigens seemed to be related to the scabies antigens. On the contrary, the Ø.B. serum showed particularly strong reaction in CRIE to the DF antigen which was partly identical with scabies antigen No. 3. Using this serum in the CRIE-tandem-experi-

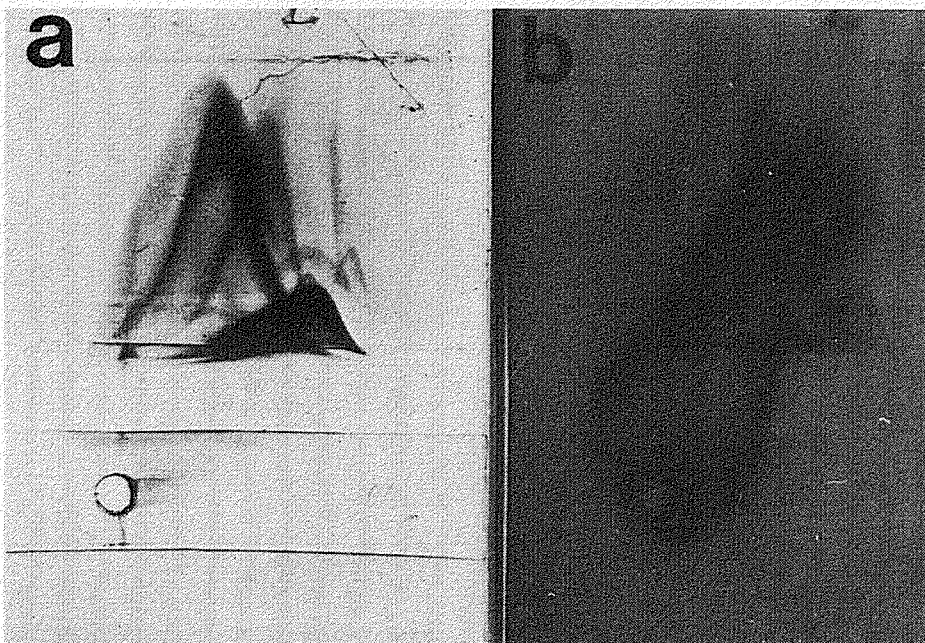


Fig. 1a. Crossed immunoelectrophoresis of a crude extract of *Dermatophagoides farinae* (DF), 90 µg d.m., against a rabbit antiserum to DF.

b. Autoradiography with labelled anti-IgE after incubation of the immunoprecipitates with pooled sera from patients with house dust mite allergy.

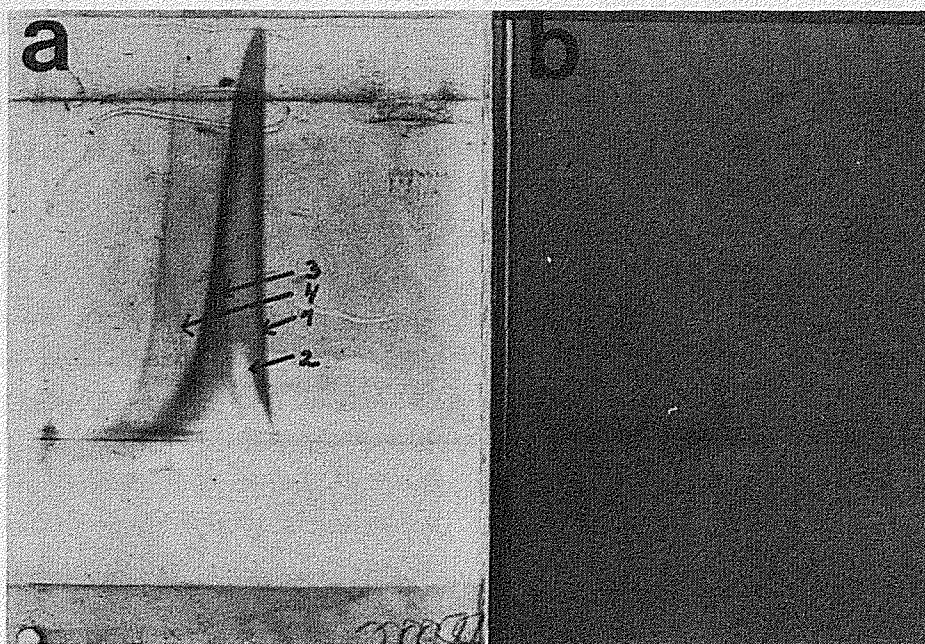


Fig. 2a. Crossed immunoelectrophoresis of a scabies mite preparation, 7 μ l, against the same antiserum as in Fig. 1a. Arrows indicate four antigens.

b. Autoradiography of the immunoprecipitates after use of the same patient serum pool as in Fig. 1b.

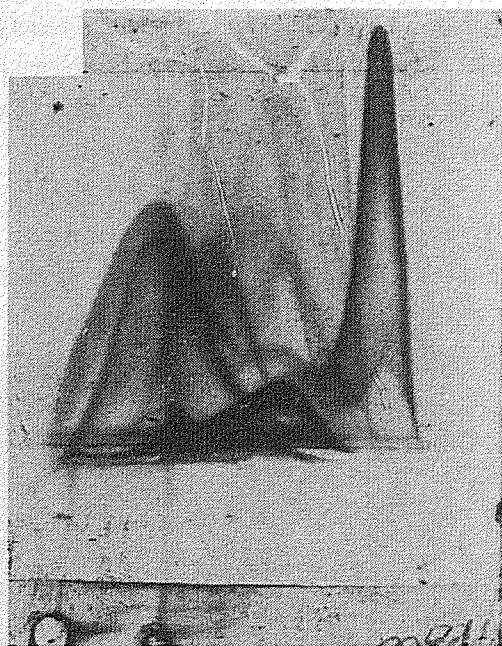


Fig. 3. Tandem crossed immunoelectrophoresis identification experiment of a scabies mite preparation, 7 μ l, (small well) and a crude *Dermatophagoides farinae* (DF) extract, 90 μ g d.m., (large well) against the same antiserum to DF as in Fig. 1a.

ment, the scabies precipitate No. 3 also gave rise to radiostaining (Fig. 4b). Thus, it is evident that human patients with house dust mite allergy can have IgE antibodies against antigens common to scabies and house dust mites.

DISCUSSION

The present results demonstrate that some rabbit antibodies evoked by immunization with house dust mites also are directed against scabies mite antigens. This indicates that there is an immunological relationship between some

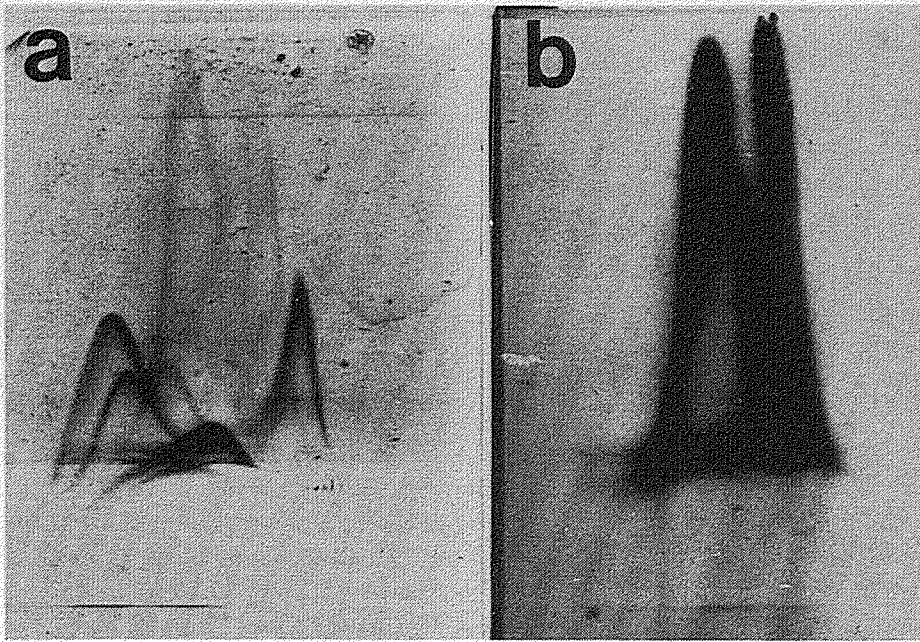


Fig 4a. Tandem crossed immunoelectrophoresis identification experiment of a crude *Dermatophagoides farinae* (DF) extract, 30 μ g d.m., (left well) and a scabies mite preparation, 2 μ l, (right well) against the same antiserum to DF as in Fig. 1.

b. Autoradiography with labelled anti-IgE after incubation of the immunoprecipitates with serum from a patient (Ø.B.) with strong IgE-antibodies to house dust mites.

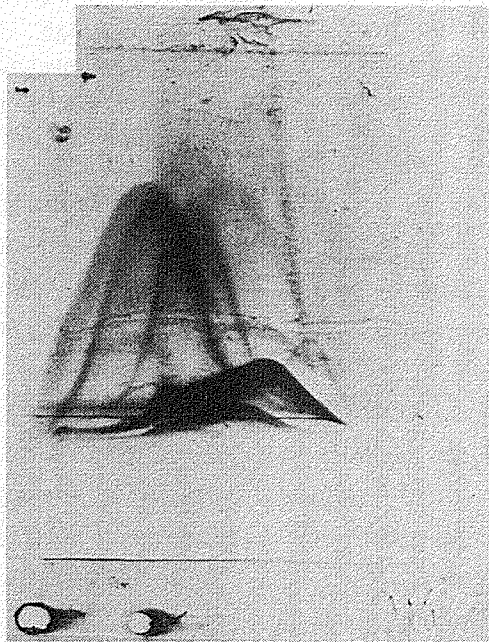


Fig. 5. Tandem crossed immunoelectrophoresis identification experiment of an extract of human epithelium preparation, 7 μ l, (small well) and a crude *Dermatophagoides farinae* (DF) extract, 90 μ g d.m., (large well) against the same antiserum to DF as in Fig. 1.

scabies and some DF antigens. This is also evident from the reaction of partial identity between immune precipitates from these antigens. Since both the scabies mites and house dust mites live on human epithelium, the antigens identified with the anti-DF serum might originate from human epithelium. However,

this possibility seems to be ruled out by the fact that no skin-derived immune precipitates could be identified with the same antisera as used with the mite preparations.

By autoradiography with labelled anti-IgE after incubation of the different immune precipitates with pooled sera from patients with

house dust mite allergy, it was not unexpected to find antibodies of the IgE class directed against DF antigens. However, in spite of the fact that no such IgE antibodies to scabies mites could be demonstrated in the serum pool, one patient with strong IgE antibodies to DF also had IgE antibodies directed against an antigen common to scabies and house dust mites. From these *in vitro* experiments and from earlier experiments *in vivo* (4) it is therefore evident that patients with house dust mite allergy and patients with scabies can develop cross-allergy. This has implications for the diagnosis of these conditions, first of all by a possible overdiagnosis of house dust mite allergy in patients who had had scabies within a few months.

Patients with house dust mite allergy do not seem to be protected against scabies infestation (3). On the contrary, more severe skin reactions can be observed in these patients during scabies infestation (3). The protective effect against reinfestation by recent scabies as suggested by Mellanby (9) is therefore likely to be the result of antibodies of classes other than IgE.

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Address:

Edvard S. Falk
Dept. of Dermatology
University of Tromsø
Tromsø
Norway

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