

Mites and house dust allergy

III. *In vitro* lymphocyte transformation and precipitating antibody to house dust and mite (*Dermatophagoides pteronyssinus*) extract in atopic and non-atopic individuals

S. ROMAGNANI, G. BILIOTTI, A. PASSALEVA and M. RICCI

Clinical Immunology Laboratory, Institute of Medical Pathology,
University of Florence

Summary

In vitro lymphocyte transformation and the precipitating antibody to house dust and mite (*Dermatophagoides pteronyssinus*) extract were studied in mite- and house-dust sensitive patients and in non-atopic individuals. House-dust extract was able to induce a significant [$2\text{-}^{14}\text{C}$]thymidine incorporation into lymphocytes from house-dust-sensitive as well as non-allergic individuals. An *in vitro* response to mite extract, even if less marked, was also seen in most of mite-sensitive patients.

By the micro-Ouchterlony and counterimmunoelectrophoresis techniques anti-dust antibodies were found in the serum of all house-dust-sensitive and non-atopic individuals tested. Anti-mite precipitins were detected in a smaller number of subjects.

Introduction

It has been suggested by several workers that mites represent the most important allergenic source of house dust (Voorhorst, Spieksma-Boezeman & Spieksma, 1964; Pepys, Chan & Hargreave, 1968; Maunsell, Wright & Cunningham, 1968; McAllen, Assem & Maunsell, 1970). In previous papers we showed a close relationship between mite and house dust allergens by comparison of skin reactions (Biliotti *et al.*, 1972) and by fractionation methods (Romagnani *et al.*, 1972). However, little is known regarding the immunologic responses to house dust and mite in man.

The main purpose of this work was to seek evidence of *in vitro* lymphocyte transformation to mite and house dust extracts in non-atopic subjects and in mite- and house-dust-sensitive patients. The presence in the serum of precipitating antibody against the same extracts has also been investigated.

Materials and methods

House dust and mite extracts

1% house dust and mite (*Dermatophagoides pteronyssinus*) extracts were provided by Diephuis Laboratory (Groningen). The extracts were extensively dialysed, ten-fold

Correspondence: Professor Mario Ricci, Istituto di Patologia Medica dell'Università di Firenze (Dir: Prof. U. Serafini), Policlinico di Careggi 50134-Firenze, Italia.

concentrated by freeze-drying and redissolved in sterile physiologic saline. Protein content of the dialysed extracts was evaluated by the Lowry method.

Selection of patients

Blood specimens were obtained from randomly selected house-dust- and mite-sensitive patients. None of the patients tested had been treated by immunotherapy. Non-atopic individuals were chosen among medical, student and technical personnel.

Skin testing

Skin tests were performed in non-atopic and atopic individuals by injecting intradermally 0.02 ml of three different dilutions of mite and house dust 1% extract (10^{-3} , 10^{-4} , 10^{-5}). The criteria for evaluating cutaneous reactions have been described in a previous work (Biliotti *et al.*, 1972).

Lymphocyte cultures

Lymphocyte cultures were performed according to a technique similar to that described by Valentine (1971). 60 ml of venous heparinized blood were obtained from each patient and transferred to screw-cap tubes. Red blood cells were sedimented at 37°C without dextran or gelatin for 1–2 hr. Leucocyte-rich plasma was collected and centrifuged. Cells were resuspended with TC 109 medium (Difco), washed twice with the same medium, counted, and lymphocytes adjusted to $0.5 \times 10^6/\text{ml}$ in TC 109 medium containing 20% of autologous plasma. The cell suspension was then divided into equal 4 ml aliquots and cultured in plastic tubes for 6 days at 37°C. Control cultures and cultures incubated with two or three different concentrations of mite and house dust extracts were performed in duplicate or triplicate. Protein concentration of mite and house dust extracts varied usually between 0.5 and 50 and 5 and 250 $\mu\text{g}/\text{ml}$, respectively. Phytohaemagglutinin-stimulated cultures were always included as a further control. 16 hr before harvesting, cultures were pulsed with 0.1 μCi [$2\text{-}^{14}\text{C}$] thymidine (specific activity 8 mCi/mm) (Radiochemical Centre, Amersham). The culture cells were then centrifuged, washed once with cold saline, twice with cold 5% TCA and methanol. The precipitates were dissolved in 1 ml of Soluene (Packard) and then mixed with 10 ml of Instagel (Packard). The counts per minute (cpm) of each sample were determined by a Nuclear Chicago Mark I scintillation counter. The mean value of duplicate determinations was considered.

Precipitating antibody

To detect precipitating antibody against mite and house dust extracts in the serum of mite- and/or house-dust-sensitive patients and non-atopic individuals the plexiglas micro-Ouchterlony (Crowle, 1958) and counterimmunoelectrophoresis (Gocke & Howe, 1970) techniques were used. To prevent non-specific reactions agar dissolved in a mixture of McIlvaine's phosphate-citrate buffer and saline, as suggested by Holford-Strevens *et al.* (1970), was also used. Concentrations of house dust extract ranged between 2.5 and 100 mg/ml (w/v). Mite extract was used at a concentration ranging between 2.5 and 250 mg/ml (w/v).

Results

Fig. 1 shows the dose-response curve obtained when lymphocytes of some mite- and house-dust-sensitive patients were cultured in the presence of different concentrations

of mite and house dust dialysed extracts. The best responses to mite extracts were noted by using $10\text{--}25\text{ }\mu\text{g}/0.5 \times 10^6$ lymphocytes but in some cases $0.5\text{ }\mu\text{g}$ were also stimulatory. House dust was able to induce a significant $[2\text{-}^{14}\text{C}]$ thymidine incorporation when a protein concentration of extract at least ten times greater was used.

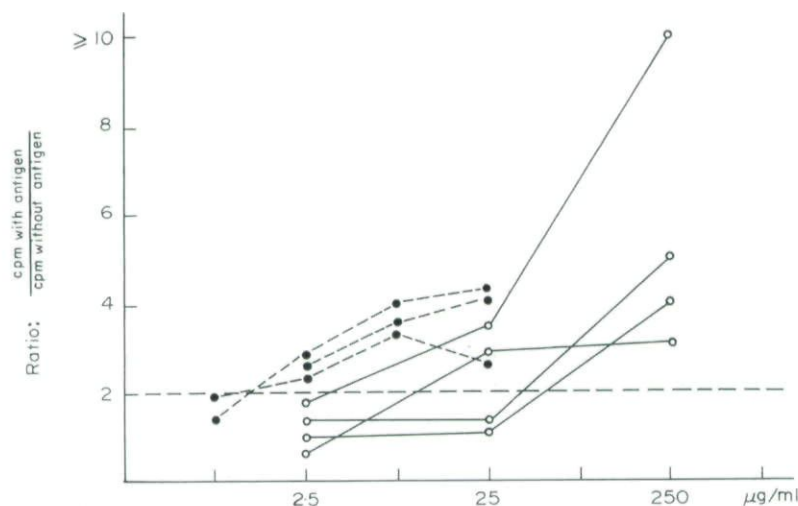


Fig. 1. Dose-dependent mite (---) and house dust (—) extract stimulation of lymphocytes from mite- and house-dust-sensitive patients. Cultures were assayed at their maximum proliferation peak, 6 days.

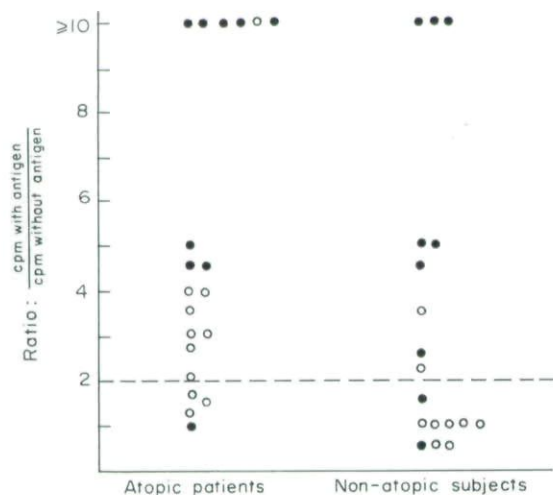


Fig. 2. Plot of specific uptake of $[2\text{-}^{14}\text{C}]$ thymidine by lymphocytes from mite- and house-dust-sensitive and non-atopic individuals. The cells were cultured for 6 days in the presence of mite (○) and house dust (●) extract, 10 and $250\text{ }\mu\text{g}/0.5 \times 10^6$ lymphocytes, respectively.

Fig. 2 shows the results obtained in the two groups of subjects examined. Mite stimulated lymphocytes from two out of nine non-atopic and seven out of ten mite-sensitive subjects showed more than double thymidine incorporation in comparison to control cultures, whereas the great majority of lymphocyte cultures from non-atopic

(seven out of nine) as well as house-dust-sensitive (nine out of ten) individuals presented highly significant levels of thymidine uptake in the presence of house dust extract.

Table 1 shows the results obtained by the micro-Ouchterlony technique. Precipitating antibodies against house dust extract constituents were found in the serum of all the house-dust-sensitive and non-atopic subjects. On the other hand, anti-mite precipitins were found in the serum of a smaller number of mite-sensitive patients and non-atopic individuals. Similar results were seen by counterimmunoelectrophoresis (Table 2). Serum anti-house dust precipitins were completely absorbed by dust antigen and were found to be present in IgG fractions on DEAE-cellulose column chromatography.

Table 1. Agar-gel micro-Ouchterlony precipitin tests in house-dust- and mite-sensitive (Group I) and non-atopic (Group II) individuals

Group	No. positive/total tested	
	House dust	<i>D. pteronyssinus</i>
I	34/34	10/24
II	20/20	5/14

Table 2. Counterimmunoelectrophoresis precipitin tests in house-dust- and mite-sensitive (Group I) and non-atopic (Group II) individuals

Group	No. positive/total tested	
	House dust	<i>D. pteronyssinus</i>
I	18/18	12/16
II	12/12	8/12

Discussion

Little information has been acquired up to now regarding the presence of cell-mediated and humoral immune responses to house dust and mite constituents in atopic and non-atopic individuals. With immunodiffusion, anti-mite precipitating antibodies were never seen in the sera of mite-sensitive subjects by McAllen *et al.* (1970). By the micro-Ouchterlony technique Holford-Strevens *et al.* (1970) showed the presence of anti-dust precipitins in the serum of many mite-sensitive and non-atopic individuals. Anti-mite (*D. farinae*) precipitins were detected in the serum of fewer subjects. Precipitins to the culture medium extract constituents were also seen. More recently Kawai *et al.* (1972) showed by counterelectrophoresis that the great majority of atopic as well as non-atopic individuals had in their serum anti-dust precipitins.

In our work we found by micro-Ouchterlony and counter-electrophoresis techniques that all house-dust-sensitive and non-atopic individuals showed precipitins to

house dust extract. Mite precipitins were detected in the serum of fewer subjects. As regards *in vitro* lymphocyte transformation, house dust extract induced high levels of thymidine incorporation into lymphocytes of almost all dust-sensitive and non-atopic individuals, whereas lymphocyte response to mite extract was less frequently seen and always less marked.

The house dust antigenic constituent(s) able to induce *in vitro* lymphocyte transformation and humoral immune responses in man have not been identified. It has been demonstrated that in house dust extracts many animal and plant constituents such as serum albumin and immunoglobulin, human dandruff, feathers, kapok, cotton linters, hay and wool can be present (Berrens & Versie, 1967). On the other hand, in mite extracts only mite and nutrient medium constituents (powdered human dandruff and dried baker's yeast) are present. In the sera of rabbits immunized with mite extracts immunodiffusion analysis revealed a major line of antigenic identity between mite, dust and human dander, but this antigen clearly was not a major allergen common to dust and mite (Kawai *et al.*, 1971). On the other hand, Dasgupta & Cunliffe (1970) found that at least one antigen was common to house dust, dust mite and human scales, but human epithelial cells were shown to possess antigens clearly distinct from those of both *D. pteronyssinus* and *D. farinae*.

Type III immune reactions have been suspected of causing the 'late' bronchial obstructive reactions due to inhalation of house dust (Booij-Noord, Orié & de Vries, 1971). Delayed- and Arthus-type cutaneous reactions have been demonstrated by skin biopsy in house-dust-sensitive patients (Bulle *et al.*, 1963). In particular delayed reactions appeared to be related to fraction S III obtained by fractionating the house dust extract on DEAE-cellulose and Sephadex G-75 (Guibert & Causse Combes, 1965). On the other hand, unfractionated house dust extract as well as S I fraction induced cutaneous reactions characterized by a predominantly polymorphonuclear infiltrate (Bulle *et al.*, 1963). These data suggest that immune responses induced by house dust are complex and probably differ according to the physico-chemical characteristics of different house dust constituents.

References

- BERRENS, L. & VERSIE, R. (1967) Antigenic relationship in atopy. II. Cross-reactions of anti house dust sera with inhalant glycoprotein allergens from different sources. *Acta Allergologica*, **22**, 347.
- BILIOTTI, G., PASSALEVA, A., ROMAGNANI, S. & RICCI, M. (1972) Mites and house dust allergy. I. Comparison between house dust and mite (*Dermatophagoides pteronyssinus* and *D. farinae*) skin reactivity. *Clinical Allergy*, **2**, 109.
- BOOIJ-NOORD, H., ORIE, N.G.M. & DE VRIES, K. (1971) Immediate and late bronchial obstructive reactions to inhalation of house dust and protective effects of disodium cromoglycate and prednisolone. *Journal of Allergy and Clinical Immunology*, **48**, 344.
- BULLE, D., BURTIN, P., VERLEY, J.-M. & KOURILSKY, R. (1963) L'allergie retardée à la poussière de maison. Etude histologique des réactions cutanées chez l'homme. *Annales de l'Institut Pasteur*, **105**, 1037.
- CROWLE, A.J. (1958) A simplified micro double-diffusion agar precipitin technique. *Journal of Laboratory and Clinical Medicine*, **52**, 784.
- DASGUPTA, A. & CUNLIFFE, A.C. (1970) Common antigenic determinants in extracts of house dust and *Dermatophagoides* species. *Clinical and Experimental Immunology*, **6**, 891.
- GOCKE, D.J. & HOWE, C. (1970) Rapid detection of Australia antigen by counterimmunoelectrophoresis. *Journal of Immunology*, **104**, 1031.
- GUIBERT, L. & CAUSSE COMBES, R. (1965) Activité allergénique des fractions obtenues par chromatographie de l'extrait de poussière de maison. *Annales de l'Institut Pasteur*, **103**, 579.

- HOLFORD-STREVEVS, V., WIDE, L., MILNE, J.F. & PEPYS, J. (1970) Allergens and antigens of *Dermatophagoides farinae*. *Clinical and Experimental Immunology*, **6**, 49.
- KAWAI, T., ARQUEMBOURG, P., SALVAGGIO, J. & MARSH, D. (1972) Detection of anti-house dust precipitins in man. *Journal of Allergy and Clinical Immunology*, **49**, 134.
- KAWAI, T., MARSH, D.G., NAGY, S.M. & NORMAN, P.S. (1971) The significance of *Dermatophagoides* allergens in house dust allergy in the Baltimore area: assay by leukocyte histamine release. American Academy of Allergy, Chicago Meeting.
- MAUNSELL, K., WRIGHT, D.G. & CUNNINGTON, A.M. (1968) Mites and house-dust allergy in bronchial asthma. *Lancet*, **i**, 1267.
- MCALLEN, M.K., ASSEM, E.S.K. & MAUNSELL, K. (1970) House dust mite asthma. Results of challenge tests on five criteria with *Dermatophagoides pteronyssinus*. *British Medical Journal*, **ii**, 50.
- PEPYS, J., CHAN, M. & HARGREAVE, F.E. (1968) Mites and house dust allergy. *Lancet*, **i**, 1270.
- ROMAGNANI, S., BILIOTTI, G., PASSALEVA, A. & RICCI, M. (1972) Mites and house dust allergy. II. Relationship between house dust and mite (*Dermatophagoides pteronyssinus* and *D. farinae*) allergens by fractionation methods. *Clinical Allergy*, **2**, 115.
- VALENTINE, F. (1971) Lymphocyte transformation: the proliferation of human blood lymphocytes stimulated by antigen *in vitro*. In *Vitro Methods in Cell-Mediated Immunity* (Ed. by B. R. Bloom and P. R. Glade), p. 443. Academic Press, London.
- VOORHORST, R., SPIEKMA-BOEZEMAN, M.I.A. & SPIEKMA, F.TH.M. (1964) Is a mite (*Dermatophagoides* sp.) the producer of the house dust allergen? *Allergie und Asthma*, **10**, 329.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.