

Temporal association between *Der pI* exposure, immediate hypersensitivity and clinical severity of eczema

A. J. W. HENDERSON, C. T. C. KENNEDY,¹ S. J. THOMPSON² & F. CARSWELL

Departments of Child Health and ¹Dermatology, Institute of Child Health, Royal Hospital for Sick Children, Bristol, and

²Department of Pathology, University of Bristol, U.K.

Uncontrolled studies of avoidance of house dust mite (*Dermatophagoides pteronyssinus*) by atopic subjects have reported clinical improvement in the severity of eczema. The aim of the present study was to examine the temporal relationship between environmental exposure to the major mite allergen (*Der pI*) and clinical disease severity in children with chronic atopic eczema. Twelve children were identified as being house dust mite-sensitive on the basis of skin prick test and RAST to *Der pI*. They were examined on two occasions with a median interval of 63 days. Clinical severity of eczema improved in nine children and deteriorated in two children during this period. *Der pI* concentration in dust from mattresses changed significantly in only three subjects and there was no correlation between changes in clinical severity and changes in environmental *Der pI* exposure. Change in RAST against *Der pI* did correlate positively with change in *Der pI* exposure ($\rho = 0.56$, $P < 0.05$) but these changes were not associated significantly with changes in eczema severity or skin test response to *Der pI*. It is concluded that the observed changes in clinical severity were unlikely to be due to immediate hypersensitivity responses to natural variations in *Der pI* concentrations in the personal environments of these subjects.

Key words: allergens; dermatitis, atopic; dust; eczema; environmental exposure; hypersensitivity, immediate.

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CLINICAL ASPECTS

Twelve children with eczema and house dust mite (*D. pteronyssinus*) sensitivity (skin test wheal: histamine wheal ratio > 1.0 and/or RAST score > 3 PRU) were studied on two separate occasions with a median interval of 63 days. Changes in clinical severity of eczema during this period were assessed using a standard scoring system. All patients were being treated for their eczema at the time of entry into the study. No changes were made to medication type or frequency of administration for the duration of the study. Immediate hypersensitivity to mite allergen was assessed using skin prick tests against histamine 1%, purified *Der pI* allergen and mite body allergens and by measuring *Der pI*-specific RAST in venous blood. The *Der pI* concentration in dust from mattresses was measured at the same time as clinical assessment. The clinical severity of eczema improved in nine subjects and deteriorated in two. This may have been due to placebo effects of medical interest in these patients or the natural history of the disease. No temporal association was demonstrated between environmental *Der pI* exposure and clinical severity of eczema in these mite-sensitive patients.

House dust mite allergens may penetrate the intact (22) or abraded (13) skin of patients with eczema. The application of high concentrations of mite allergen to the skin of sensitized subjects may produce characteristic lesions of eczema (16). Dilute mite allergen, in a concentration approximating assumed natural exposure, may also precipitate eczema on previously uninvolved skin of mite-sensitized subjects (17). Avoidance of mite allergens therefore might be expected to have a beneficial effect on eczema severity in such subjects. Uncontrolled studies of house dust removal by patients with eczema have reported improvements in disease severity (1, 23), assumed to be due to a reduction in mite allergen exposure. However, vacuuming is not an efficient method of removing mites (2) and, as environmental allergen concentrations were not measured, this assumption remains speculative. Admission of mite-sensitive patients to the relatively mite-free hospital environment has also been reported to improve eczema severity (21) but, without data from control subjects, a placebo effect of hospitalization cannot be discounted.

The aim of the present study was to examine the relationship between clinically observed variations in the severity of eczema and natural variation in mite allergen exposure occurring in the homes of a group of mite-sensitive children.

SUBJECTS AND METHODS

The study was approved by the ethics committee of Bristol and Weston Health Authority. Nineteen children who attended the dermatology out-patients' clinic at Bristol Children's Hospital agreed to enter the study. All 19 subjects had a diagnosis of atopic eczema according to the clinical criteria described by Hanifin (14). Skin prick tests and radioallergosorbent test against specific house dust mite allergens were performed on each child to determine which of them were mite sensitive.

Skin tests were carried out on the ventral surface of the left forearm using the prick test technique described by Pepys (18). Solutions of 1% histamine, 20 µg/ml *Der pI* and 20 µg/ml *D. pteronyssinus* body allergens in phosphate buf-

fered saline (PBS) were used. PBS alone acted as a negative control. *Der pI* solution was prepared by chromatofocussing and gel-filtration as previously described (8). Mite-body solution from a live culture of *Dermatophagoides pteronyssinus* was prepared by aqueous extraction in PBS followed by immunoabsorption using rabbit anti-*Der pI* on cyanogen bromide activated Sepharose 4B (Pharmacia Ltd) columns to remove any contaminating *Der pI*.

The early (15 min) and late (4–6 h) responses were measured following skin prick test. The wheal response at 15 min was measured in two perpendicular dimensions and the mean wheal diameter calculated. The mean wheal response to mite allergens was expressed as a ratio of the mean wheal response to 1% histamine. A ratio of 1.0 or greater was regarded as indicating mite sensitivity.

The late response was measured by examining the skin test sites at 4–6 h and tracing the area of erythema onto graph paper. The area of the response was then calculated. The skin test solutions were administered to one known mite-sensitive subject (F.C.) prior to the study. The mean late-phase response of this subject was 218.5 mm² with a coefficient of variation of 7%. However, no late phase reactions were seen in any of the study subjects.

Radioallergosorbent test (Phadebas RAST) was performed on a sample of venous blood taken from each subject prior to skin testing. The specific anti-*Der pI* immunoglobulin E concentration was measured using discs coupled with purified *Der pI*. A RAST score of 3 PRU or greater was used to indicate the presence of mite-sensitivity.

Twelve of the 19 subjects enrolled in the study were mite-sensitive based on the above criteria for skin test and RAST. Their median age was 10 years 1 month (4 y 3 m – 17 y 2 m), there were 10 females and 2 males and the median duration of their eczema was 9 years 6 months. All 12 patients were being treated with topical corticosteroids on entry into the study. The treatment regimen on entry was continued with no alterations for the duration of the study.

The clinical severity of eczema was assessed using a standard scoring system as described by

Heddle et al. (15). The body's surface was divided into 20 equal areas and each was scored from 0 (no involvement) to 3 (severe involvement) for each of three features; erythema, skin damage and lichenification. The severity score was derived by adding the 3 scores for all 20 areas to produce a maximum of 180 points. The extent score was defined as the total number of areas with any involvement, i.e. a score for that area of 1 or more, with a maximum score of 20 points. The scoring system was performed in duplicate by independent observers (A.J.W.H. and C.T.C.K.) on six subjects to assess inter-observer repeatability. There was agreement between the scores measured by different observers. For the severity score the limits of agreement (3) were +1.6 to -9.6 points with a mean difference of -4 points and for the extent score the limits of agreement were +1.2 to -3.8 points with a mean difference of -1.3 points. The correlations between observer's scores were $r = 0.97$ ($P < 0.01$) for severity and $r = 0.92$ ($P < 0.01$) for extent.

Each patient's home was visited by one of us (A.J.W.H.) on the same day as the clinic visit to re-examine the skin test sites for late-phase cutaneous reactions and to obtain a sample of dust to measure *Der pI* concentration.

The dust was collected from the mattress of the study subject's bed by vacuuming an area of 600 cm² for 120 s using a pooter pot as described by Blythe (4). An aqueous extract (PBS) was made of the weighed dust sample and the *Der pI* concentration was measured using a double antibody inhibition radioimmunoassay (9) with rabbit anti-*Der pI* and goat anti-rabbit IgG. Known concentrations of purified *Der pI* in PBS were used as controls. The coefficient of repeatability (3) of the measurement was assessed between-sites, by taking paired samples on the same day from 8 mattresses, and between-assays, by performing duplicate immunoassays on 34 dust samples. The composite coefficient of repeatability was ± 72.2 µg/g dust.

Each subject was re-examined after a median interval of 63 days (21 d - 133 d). A second home visit was made to collect a repeat dust sample. The first and second dust samples were taken from opposite halves of the mattress in

comparable positions. This was done to avoid interference of the first sampling procedure on the *Der pI* concentration in the second sample. Changes in *Der pI* concentrations between the two visits were regarded as significant only if they exceeded the coefficient of repeatability of the assay.

The variables measured in this study were not normally distributed and non-parametric statistical analyses were used. Relationships between the variables were examined using Spearman's rank correlation, and results are expressed as the rank correlation co-efficient, rho.

RESULTS

Twelve mite-sensitive children were studied. The median severity of their eczema was 24 points (7-69) and the median extent 9 points (4-18). The mean skin test response to mite body allergen (1.04 (0.87 SD)) was higher than the mean response to *Der pI* (0.79 (0.45 SD)) in these subjects but the difference was not significant (paired t-test).

Between the two study visits the clinical severity of eczema improved in nine subjects, two became worse and one did not change. The median decrease in severity scores in the nine subjects who improved was 5 points (1-27). The increase in severity scores of the two subjects who became worse was 5 points in each case. Eczema extent scores decreased in nine subjects (median 2 points (1-9)) and increased in three subjects (median 1 point (1-2)). There was a positive correlation between changes in eczema severity and extent in these patients (Spearman's rho = 0.78, $P < 0.01$). There was also a positive correlation between the magnitude of the change in severity and the interval between assessments but this was not significant (Spearman's rho = 0.53).

The mean *Der pI* concentration in dust from the patient's homes was 38.4 µg/g (54.2 µg/g SD) on the first visit and 34.1 µg/g (55.3 µg/g SD) on the second. There was considerable variation in the magnitude of change of *Der pI* concentrations between the two visits from 0.2 µg/g to 160 µg/g but in only three cases did it exceed the coefficient of repeatability of the

Table 1

Spearman's rank correlation coefficients (rho) between eczema severity, *Der pI* exposure and skin test/RAST to *Der pI* in 12 mite-sensitive subjects

	Severity score	Extent score	<i>Der pI</i> exposure
Extent score	0.91**		
<i>Der pI</i>	-0.10	-0.28	
Skin test	-0.04	-0.06	-0.42
RAST	0.42	0.55	0.14

Key: ***P* < 0.01

measurement. These three subjects are described in more detail below.

The change in *Der pI* concentration in dust from the homes of patients whose eczema severity improved (mean + 6.2 µg/g (77.45 µg/g SD)) was not significantly different from that in those whose eczema worsened (mean -58.7 µg/g (85.1 µg/g SD)). There was no significant correlation between the magnitude of the change in *Der pI* concentrations and the interval between measurements (Spearman's rho = -0.16).

The correlations between initial severity of eczema, *Der pI* concentration in house dust and immediate hypersensitivity responses to *Der pI* are demonstrated in Table 1. The positive correlation between eczema severity and extent and RAST to *Der pI* in these mite-sensitive patients is not significant. There is no relationship between *Der pI* exposure as measured in the mattress dust and either eczema severity or immediate hypersensitivity to *Der pI*.

Correlations between changes in these parameters from visit 1 to visit 2 are demonstrated in Table 2. Although *Der pI* concentration did not correlate with RAST initially, there is a positive correlation between changes in *Der pI* exposure and RAST to *Der pI* in these patients' sera. The apparent initial relationship between eczema severity and RAST is not maintained.

Three individual subjects are noteworthy in having significant changes in *Der pI* exposure between their two visits. In two of these subjects there were also moderate to large changes in RAST and clinical severity over the same period. These data are summarized in Table 3. All three subjects had an improvement in clinical

Table 2

Spearman's rank correlation coefficients (rho) between changes in eczema severity, *Der pI* exposure and skin test/RAST to *Der pI* in 12 mite-sensitive subjects

	Severity score	Extent score	<i>Der pI</i> exposure
Extent score	0.78**		
<i>Der pI</i>	-0.08	-0.02	
Skin test	-0.10	-0.34	0.19
RAST	-0.52	-0.46	0.56*

Key: **P* < 0.05; ***P* < 0.01

severity and, in two cases, this was associated with a decrease in *Der pI* concentration and a decrease in RAST to *Der pI*. However, the subject with the greatest decrease in severity had a significant increase in both *Der pI* exposure and serum RAST to *Der pI*.

DISCUSSION

In this group of house dust mite-sensitive children we have not demonstrated a temporal correlation between exposure to the major mite allergen, *Der pI*, and the clinical severity of eczema. Changes in *Der pI* concentrations in dust from the homes of these patients were positively correlated with changes in *Der pI*-specific RAST in their sera but these changes did not relate to changes in either clinical severity of eczema or skin test response to *Der pI*.

A possible explanation for our observation is that the natural variations which occurred in *Der pI* concentrations were of insufficient magnitude to induce changes in clinical response. There are no reliable data to indicate how much mite antigen needs to be applied to the skin to produce an eczematous response. Sixty micrograms of *Der pI* have been demonstrated to increase the area of existing eczema in sensitized subjects but not to produce a significantly greater area of dermatitis than diluent control on uninvolved skin (17). A concentration of 2 µg *Der pI*/g dust has been proposed as a risk factor for sensitization of patients with asthma (19). It may be that in the present study the changes in *Der pI* which were observed failed to reach a critical minimum threshold for clinical improvement to occur, or that they occurred on an insensitive

portion of the patients' dose response curves to the allergen. However, over a large range of *Der pI* concentrations (0.4–175.0 µg/g), no relationship with eczema severity was demonstrated.

We chose to adopt a natural historical approach to this study as opposed to an intervention study in which measures to reduce house dust mite allergen exposure were attempted. It is difficult to reduce house dust mite allergen significantly more than may occur by natural variation. Previous studies of house dust mite avoidance by patients with eczema have relied upon vacuum cleaning to remove mites (1, 23) but this technique is ineffective in removing both mites and mite-allergen from carpets (4) and, even when performed intensively by trained personnel, may reduce the mite content of carpets by approximately 16-fold (7) whereas natural variations in mite allergen content of house dust from the homes of patients with asthma of up to 20-fold have been demonstrated (20). The maximum change in *Der pI* concentration observed in the present study was 6-fold.

It is also possible to reduce house dust mite allergen content of carpets by using acaricidal sprays. However, even the latest method of mite allergen removal using benzylbenzoate has failed to produce a significant decrease in the *Der pI* concentration of house dust from mattresses although it reduces allergen concentration in carpets in the short term (24). We reasoned that the mattress was the logical sampling site as it is the greatest source of house dust mite in the home (6) and, as house dust mite sensitization in eczema is likely to occur through the skin (8), it is the site of greatest contact between dust and patient.

A further factor which mitigated against the use of an intervention study was the difficulty in excluding a placebo effect in the group of patients who received mite prophylaxis. In the present study, despite the fact that we carried out no direct manipulation of each patient's home environment, the majority of patients displayed a clinical improvement in their eczema, and we cannot dismiss the possibility of a placebo-effect of our interest in these subjects. Alternatively, participation in the study may have persuaded the families to adopt more vigorous cleaning regimens at home but we did not observe an overall decrease in *Der pI* concentrations if this was the case. Placebo effects have not been considered in previous studies which reported improvement in eczema when patients carried out mite removal techniques at home (1, 23), or when they were admitted to hospital (21), but such effects are probably important as controlled trials of anti-mite measures in children with asthma have failed to show symptomatic differences between treatment and control groups (5, 12).

The lack of relationship between clinical severity and *Der pI* exposure in the present study may be a reflection of the relatively greater importance of house dust mite body allergens in triggering skin allergy (8), but we were unable to measure the concentrations of these allergens. However, we observed no significant differences in the skin test responses of this group of patients to equal concentrations of *Der pI* and mite body allergens. The other possible explanation is that there was a temporal dissociation between the measured exposure to house dust mite allergen and the expression of clinical response to this allergen. This would seem likely as eczema represents a cell-mediated, late-phase reaction (10, 11) rather than

Table 3
Clinical severity of eczema, *Der pI* concentration in mattress dust and serum RAST to *Der pI* in three subjects with significant changes in *Der pI* exposure

Subjects	Severity score (units)		<i>Der pI</i> exposure (µg/g)		RAST (PRU)	
	visit 1	visit 2	visit 1	visit 2	visit 1	visit 2
1	16	4	15.0	175.0	4	> 4
2	25	20	144.8	26.0	4	3–4
3	25	21	130.8	2.0	4	3

an immediate hypersensitivity response, and we measured *Der pI* exposure and clinical severity contemporaneously. Although we did not design the study specifically to address this point, we did attempt to document late-phase cutaneous responses to mite allergens which we observed in one adult volunteer but in none of our study population. However, patch tests or intradermal tests may have been more useful in this regard than our skin prick tests and this important aspect should be examined in future studies.

The results of our study do not support the existence of a direct, temporal correlation between the amount of house dust mite allergen in dust from the mattress and clinically observed, spontaneous variation in eczema severity, although mite allergen may mediate its effect through delayed hypersensitivity reactions which mask such a relationship. Future clinical studies of the efficacy of mite removal techniques in eczema should be controlled for the achieved reduction in mite numbers and mite allergen and for placebo effects of medical intervention in this multi-factorial disease.

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Address:

Dr. A. J. W. Henderson

Department of Respiratory Medicine

Princess Margaret Hospital for Children

GPO Box D184

Perth

Western Australia 6001

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