

House dust mite sensitivity: interaction of genetics and allergen dosage

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Summary

Pairwise analysis of siblings from 21 families showed that house dust mite (HDM) sensitive children were exposed to higher concentrations of *Der p* I allergen in their mattress ($P=0.005$) and bedding ($P=0.04$), but not bedroom floor ($P=0.33$), than their atopic sibling who was not sensitive to HDM antigens. There was no difference in the exposure to HDM numbers/100 mg of dust in the mattress ($P=0.61$) or bedroom floors ($P=0.09$). In contrast, pairwise analysis of siblings from 15 families showed that HDM sensitive children were not exposed to significantly different concentrations of *Der p* I in the mattress ($P=0.96$), bedding ($P=0.11$) or bedroom floor ($P=0.70$) nor HDM numbers/100 mg of dust in the mattress ($P=0.12$) and bedroom floor ($P=0.98$) than their non-atopic siblings. These findings were identical when absolute allergen load was compared in these pairs. Genetic linkage studies in these families suggest the tendency to atopic IgE responses is conferred by a putative atopy locus on chromosome 11q. These results together suggest that differences in allergen levels in beds, among siblings with a comparable genetic tendency to atopy, play a significant role in determining the development of HDM allergy.

Clinical and Experimental Allergy, Vol. 22, pp. 205–211. Received 15 May 1991; revised 3 September 1991; accepted 4 September 1991.

Introduction

It is generally accepted that atopy is defined as the familial predisposition to producing immunoglobulin E (IgE) to one or more of a range of allergens, predominantly inhaled and present in low levels in the environment [1–3]. Numerous studies have demonstrated that both genetic and environmental factors are important in determining sensitization to these allergens. Comparable concordance rates in monozygotic and dizygotic twin studies suggest that the environment is important in determining an atopic individual's particular sensitivities [4]. A recent genetic linkage study using immunological (IgE) criteria to assign atopy phenotype has identified a putative 'atopy' locus on chromosome 11q [5]. This has been confirmed in a study of 64 young and representative nuclear families in which the 11q atopy locus was shown

to underly atopy in these families [6]. Thus, individuals with this inherited propensity to exaggerated IgE responses may become sensitized to a range of common environmental allergens following exposure.

House dust mite species (especially *Dermatophagoides pteronyssinus*) are the source of the major sensitizing allergens underlying asthma in atopic individuals in Britain. Studies have suggested that magnitude of exposure to house dust mite (HDM) allergens influence specific IgE responses to them [7–18]. However conclusions on potential confounding environmental effects and/or interaction with a genetic tendency to atopy have been limited as all of these studies compare unrelated individuals and most have used either no control group [9–11], controls of unknown atopic status [12], or controls who were not sensitive to HDM allergens but had poorly documented IgE responses to other allergens [13–15].

Therefore, we have investigated the importance of the magnitude of exposure to HDM allergen by comparing allergen levels in HDM sensitive children with their

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siblings. Comparisons were made between HDM sensitive children and their atopic non-HDM sensitive siblings in 21 families and between HDM sensitive children and their non-atopic siblings in 15 families. Segregation and genetic linkage analysis in these families, using IgE criteria to assign atopic status, has confirmed that the children's general atopic predisposition is dominantly inherited and conferred by the 11q atopy locus [ref. 6 and unpublished observations]. We have carefully documented the duration of time that sampled sites were in use since birth and we have used a recommended standardized method of dust sampling [19,20] to determine the differences in exposure to HDM allergens in terms of both mite numbers/100 mg of dust and *Der p* I allergen level (as concentration $\mu\text{g/g}$ of dust and load $\mu\text{g/sample}$).

Subjects and methods

Subjects

Families with young atopic children were targeted for this study. Children under 10 years of age who reported symptoms suggestive of atopic asthma, rhinitis or eczema were identified (probands) from allergy clinic and asthma clinic records or from responses following a media appeal. The families of these children were recruited if both parents and at least two children (including the proband) were available for study. The members of each family were tested and samples collected from their home during one visit between the months of July and September, 1988 or between the months of January and March, 1989.

IgE phenotyping

Skin prick testing was carried out with a negative control and the following purified and dialysed antigens suspended in a 50% glycerine solution: *D. pteronyssinus*, mixed grasses, cat dander, dog dander, mixed trees, *Alternaria*, *Cladosporium* and *Aspergillus* (Dome/Hollister-Steir, Spokane, Washington, U.S.A.). Total and specific IgE titres were determined by a solid-phase immunoassay (Phadezym IgE EIA and Phadezym RAST, Pharmacia Ltd, Milton Keynes, U.K.) using *D. pteronyssinus*, mixed grasses, mixed moulds and mixed epithelium antigen discs. We designated children as atopic according to our previous criteria [5,6]; a skin weal of 2 mm or more than control to any antigen, or a RAST score of 1 or more (>0.35 RU/ml) to any antigen, or an elevated total IgE (geometric mean plus one standard deviation as recommended by Pharmacia) [21,22]. An individual was designated as sensitive to house dust mites (HDM-atopic) if they had a RAST score of 1 or more and/or a skin prick weal of 2 mm or more than control, to *D. pteronyssinus*. Phenotyping of children was determined prior to dust

analysis and identified families with sibling pairs comprising of HDM-atopic children and their atopic non-HDM sensitive or non-atopic siblings.

Determination of mite numbers and quantification of *Der p* I

Resting dust was sampled (by R.Y.) from an area of 1 m² for 2 min using a Medivac vacuum cleaner 1000 W (Medivac Ltd, Cheshire, U.K.) [20]. Particles were trapped on a 0.5 μm glass fibre filter paper (Whatman Products Ltd, Maidstone, U.K.). Each filter was halved for separate determination of mite species/numbers and levels of *Der p* I (see below). Sites sampled were childrens' mattresses (upper surface), bedding (upper surface) and childrens' bedroom floor (play area/beside bed). Due to small samples, bedding dust was analysed for *Der p* I levels only. The length of time the children had used each sample site was noted. Families were unaware of the sites to be sampled and were instructed not to alter their normal cleaning activities.

Analysis of dust samples (by B.H. and T.M.) was done blind to phenotype data. For mite analysis dust was scraped from the filter, large particles removed, and 100 mg of fine dust weighed and soaked in 80% ethanol. Mites were extracted and counted by the flotation technique [23] and the following species identified by light microscopy [24,25]: *D. pteronyssinus*, *D. farinae*, *D. microceras* and *E. maynei*. Other mite species, present in low numbers ($<5\%$ total mites/sites), were collectively called 'other' and added to the above species to give a total mite count for each site. Dust samples for *Der p* I analysis were weighed, gently mixed with 10 ml of phosphate-buffered saline for 3 hr and after standing overnight at 4°C supernatants collected for *Der p* I-specific ELISA assay [26]. The standard calibration curve ranged from 0.5–250 ng *Der p* I/ml and the within assay coefficient of variation was 6.7% at the mid-point. The absolute level of *Der p* I for each sample was determined and then divided by the weight to give the concentration of *Der p* I in $\mu\text{g/g}$ of dust.

Detailed analysis of the mite species present in these homes, published elsewhere, confirms the predominance of *D. pteronyssinus* as the major source of sensitizing mite allergens accounting for over 90% of total mites present in most sites [27]. Resting dust is the ultimate source of respirable particles such as those containing *Der p* I (a major mite allergen from *D. pteronyssinus*), the levels of which have been shown to be highly correlated with levels in airborne samples when collection methods are carefully standardized [28].

Data on mite numbers/100 mg of dust and *Der p* I levels for each site sampled were logarithmically transformed

for comparisons between sibling pairs. Comparisons for all sibling pairs were not possible for some sites due to sharing of these sites by siblings. Data from the two sampling periods (July–September and January–March) were combined on the basis that the comparisons were between sites sampled at the same time. We used a two-tailed paired *t*-test to calculate the magnitude of the differences between pairs, the 5% level being taken as significant.

Results

Sibling pair IgE responses

Of the 36 families studied, 21 included at least two atopic children, one of whom was sensitive to HDM allergens (HDM-atopic) and the other sensitive to other allergens but not HDM allergens (non-HDM atopic) (Comparison A). The remaining 15 families included one HDM-atopic child and one non-atopic sibling (Comparison B).

Comparison A. HDM-atopic children comprised 10 boys and 11 girls with ages ranging from 7–10 years (mean age 8.0 years). Twenty of the 21 (95%) had an elevated total IgE and all had positive RAST to *D. pteronyssinus*, 12/21 (57%) were also *D. pteronyssinus* skin test positive and 14/21 (67%) were skin and/or RAST positive to other allergens. All were reported to suffer from two or more of either asthma, rhinitis or eczema. Fourteen boys and 7 girls with ages ranging from 2.5 to 14 years (mean age 7.0 years) were assigned as atopic but non-HDM sensitive. Nine were assigned on the basis of positive skin and/or RAST tests (to allergens other than HDM) and 12 on the basis of an elevated total IgE. In this latter group, nine were more than two standard deviations above the mean for their age (highly predictive for subsequent development of atopic symptoms) [29] and two of the remaining three suffered asthma and rhinitis. Thirteen of the 21 children (62%) were reported to suffer from one or more of either asthma, rhinitis or eczema. All 21 sibling pairs had at least one atopic parent.

Comparison B. HDM-atopic children comprised eight girls and seven boys with ages ranging from 2–10 years (mean age 7.1 years). Thirteen of the 15 (87%) had an elevated total IgE and all 15 were RAST positive to *D. pteronyssinus*, 14 (93%) were skin test positive to *D. pteronyssinus*, 11/15 (73%) were sensitive to other allergens on skin and/or RAST testing and 14 reported to suffer from two or more of either asthma, rhinitis or eczema. The non-atopic controls consisted of seven girls and eight boys with ages ranging from 1 to 11 years (mean age 6.7 years). These 15 non-atopic children were by definition, RAST and skin prick test negative and had a

normal total IgE. Three of these children were reported to suffer from asthma.

A paired *t*-test comparison of the siblings in both comparisons A and B showed that there was no significant difference in their ages or duration of usage of the sites sampled.

Sibling pair exposure

Comparison A. The concentration of *Der p* I $\mu\text{g/g}$ of dust in both the mattress ($n=20$ pairs) and bedding ($n=8$ pairs) of the HDM-atopic children were significantly higher than their non-HDM atopic siblings ($P=0.005$ and $P=0.04$ respectively). This is shown in Fig. 1a, b where 15/20 pairs and 7/8 pairs fall below the line of equal *Der p* I concentration (i.e. *Der p* I $\mu\text{g/g}$ exposure is higher for the HDM-atopic children) in the mattress and bedding respectively. When comparing the 9/20 sibling pairs in which the non-HDM atopic child has specific IgE to the other aero-allergens tested (not an elevated total IgE alone), 8/9 fall below the line of equal *Der p* I concentration in the mattress ($P=0.03$). In the remaining sib pairs in which the non-HDM sibling had an elevated total IgE alone, two pairs were on the line 7/9 were below the line of equal concentration ($P=0.02$). Six of the 20 non-HDM atopic children were exposed to concentrations above the proposed 'very high risk' concentration for sensitization of 10 $\mu\text{g/g}$ of *Der p* I of dust (Fig. 1a). Four of the 20 HDM-atopic children were exposed to *Der p* I concentrations less than 10 $\mu\text{g/g}$ of dust and three exposed to less than 2 $\mu\text{g/g}$. A comparison in the bedding was possible for only five sibling pairs of the 9/20 described above and showed 4/5 pairs fall below the line of equal concentration in the bedding ($P=0.31$). We found no difference in the levels of *Der p* I concentration in the bedroom floor ($n=16$) with 11/16 pairs below the line of equal exposure concentration ($P=0.33$). When the same sibling pairs were compared using *Der p* I $\mu\text{g/sample}$ as a measure of exposure (collected over a standardized area and time) the differences were comparable (mattress $P=0.02$, bedding $P=0.04$ and bedroom floor $P=0.21$). Comparison of HDM numbers found in both mattresses and bedroom floors for these pairs, revealed no difference ($P=0.61$ and $P=0.09$ respectively). This is shown in Fig. 2a, b where 8/17 pairs and 10/16 pairs respectively fall below the line of equal mite numbers.

Comparison B. There was no difference in the levels of *Der p* I concentration between the HDM-atopic and non-atopic sibling pairs in the mattress (Fig. 3a) with 8/15 pairs below the line ($P=0.96$), in the bedding (Fig. 3b) with 2/8 pairs below the line ($P=0.11$) or in the bedroom floor ($P=0.70$). Seven of the 15 HDM-atopic children

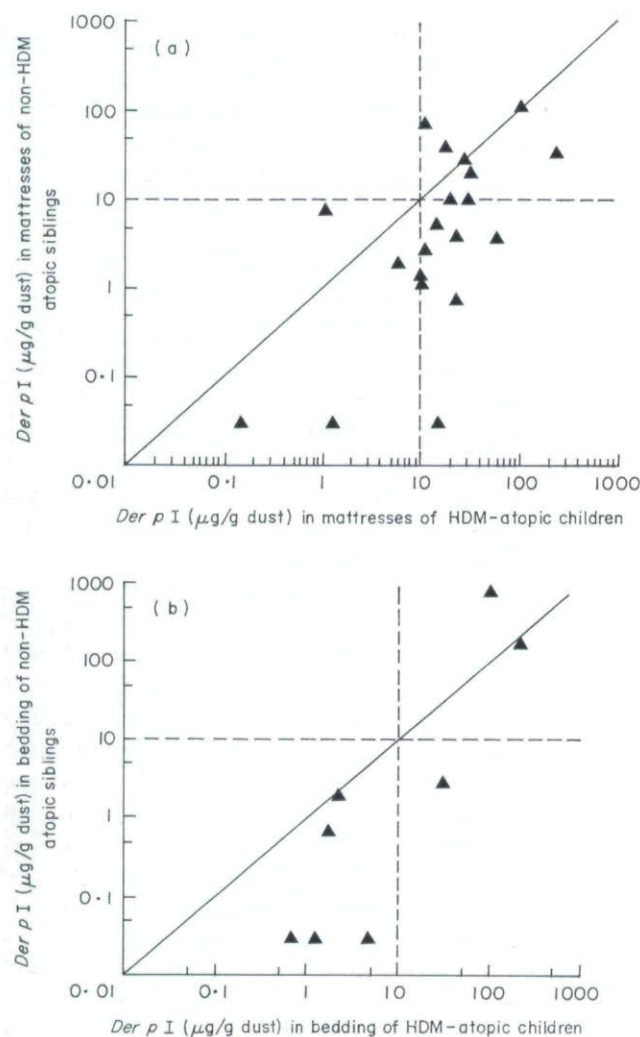


Fig. 1. Concentration of *Der p I* in (a) the mattresses and (b) the bedding of HDM-atopic children plotted against those of their non-HDM atopic sibling (20 and eight pairs respectively). The diagonal line joins points of equal *Der p I* concentration. The perpendicular lines mark the concentration of 10 µg of *Der p I*/g of dust associated with very high risk of HDM sensitization.

were exposed to levels of *Der p I* below 2 µg/g of dust in the mattress (Fig. 3a). Again comparable results were seen when comparing *Der p I*/sample in these pairs (mattress $P=0.53$, bedding $P=0.49$ and bedroom floor $P=0.53$). No differences in exposure to mite numbers in the mattress ($P=0.12$) and bedroom floor ($P=0.98$) were observed in these pairs.

Forty-eight per cent of the sampled sites had been present in the environment of the children for 75% of their lives and 39% of sites had been present since birth. The range of levels of *Der p I* concentration (and absolute) in the two HDM-atopic groups were comparable for each site sampled (Figs 1a and 3a).

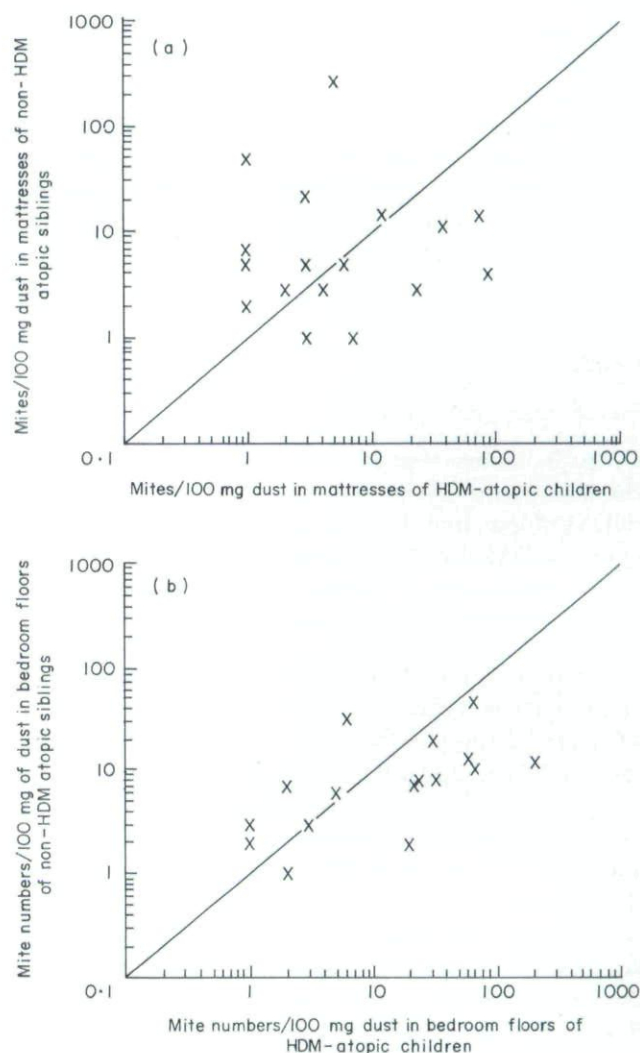


Fig. 2. Mite numbers per 100 mg of dust in (a) the mattresses and (b) the bedroom floors of HDM-atopic children plotted against those of their non-HDM atopic sibling (17 and eight pairs respectively). The diagonal line joins points of equal mite numbers.

Discussion

In a retrospective study we have investigated the importance of the magnitude of exposure to house dust mite by comparing levels of mite numbers and allergen dose from important domestic sites in use by young HDM sensitive children and their siblings. Comparisons were made between HDM sensitive children and their atopic non-HDM sensitive siblings in 21 families and between HDM sensitive children and their non-atopic siblings in 15 families. We have demonstrated that the sites have been in use by the children for a significant part of their lives. The HDM-atopic children from 21 families had significantly higher (although widely ranging) levels of *Der p I* in their

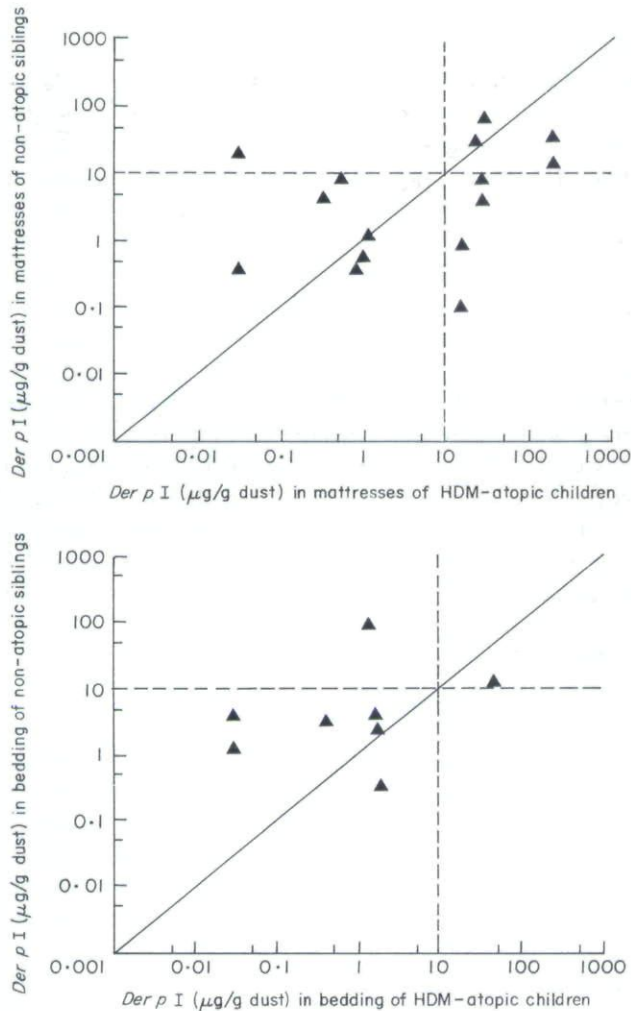


Fig. 3. Concentration of *Der p I* in (a) the mattresses and (b) the bedding of HDM-atopic children plotted against those of their non-atopic sibling (15 and eight pairs respectively). The diagonal line joins points of equal *Der p I* concentration. The perpendicular lines mark the concentration of 10 µg of *Der p I*/g of dust associated with very high risk of HDM sensitization.

mattress and bedding but not bedroom floor, compared to their non-HDM atopic siblings. However, in 15 other families the levels of mite numbers and *Der p I* were not significantly different in any sites in use by HDM-atopic children compared with their non-atopic siblings. In a previous study genetic linkage analysis in these families suggest the tendency to atopic IgE responses is conferred by a putative atopy locus on chromosome 11q. We conclude from our results that the magnitude of HDM allergen exposure, particularly in beds, is of primary importance in determining specific IgE responses in children with a comparable genetic predisposition to atopy.

Previous studies investigating HDM exposure and sensitization have used unrelated individuals as controls [12–18]. Our use of sibling pairs provides more stringent controls for confounding effects, both environmental (e.g. cultural or socio-economic) [10] such as parental smoking and also genetic [5,6,30], which may influence HDM exposure (the former only) or response to HDM allergens (both). We have demonstrated that both the HDM-atopic children and their non-HDM atopic siblings (Comparison A) have atopic IgE responsiveness and are drawn from families in which genetic studies show that this immunological phenotype is linked to chromosome 11q [5,6]. Sensitization to HDM allergens occurs in individuals who are atopic IgE responsive—with 25/36 (69%) showing IgE responses to other common inhaled allergens. Our results show that the HDM sensitized child is exposed to higher levels of HDM allergen in their beds (mattress and bedding) than their non-sensitized atopic sibs, even when the latter is divided into those with specific IgE responses and those with an elevated total IgE alone. This result supports those of other studies suggesting that allergen exposure increases risk of sensitization to HDM allergens. However, mite numbers were not significantly different in any site between these pairs.

In contrast, non-atopic children (those with no demonstrable atopic IgE responsiveness) and their HDM-atopic siblings were exposed to comparable levels of HDM allergen (Comparison B). These sibling pairs were also from families in which our immunological phenotype for atopy is linked to 11q. These results suggest that the non-atopic children in these families did not mount an IgE response to HDM, despite exposure to HDM allergens comparable with their sensitized siblings, because they are not genetically predisposed to atopy. This would mean studies that have used non-atopic children as controls can not determine the effect of allergen dose on sensitization reliably as non-atopics would not expect to become sensitized at natural levels of exposure (as shown by this study). However, the non-atopic control group from Comparison B is useful in showing that the differences in HDM allergen levels observed in Comparison A are unlikely to be a consequence of HDM sensitization (i.e. secondary rather than primary phenomenon). This is because the *Der p I* levels in the HDM-atopic children in Comparison B were not significantly higher than the non-atopic control group.

Despite the retrospective design of this study an exposure dosage effect on sensitization was found for allergen dosage. We believe this is due to several factors. The importance of *Der p I* allergen was established by confirming the predominance of *D. pteronyssinus* as the most common mite in the homes tested and therefore a likely source of sensitizing mite allergens in these children

[27]. The sites sampled have been in use by the children for a significant part of their lives. Sporik *et al.* reported comparable concentrations of *Der p I* in homes sampled 11 years later [17]. Mite allergens, unlike mites themselves, are associated with particles of respirable size. In addition, the factors contributing to mite and allergen levels are likely to be sufficiently different to explain the poor correlation we observed between the two (unpublished observations) reflected in the differences in their seasonal variation [19]. The allergen exposure dosage effect on HDM sensitization was found for both concentration and absolute load of *Der p I*. The former is useful for comparative purposes and when dust sampling is not standardized over area and time. We chose to compare allergen load in order to account for the huge variation in weight of dust from the sites sampled. The results of this study suggest that both concentration and absolute load provide estimates of exposure relevant to HDM sensitization.

Although the HDM-atopic children had both higher absolute levels and concentrations of *Der p I* in their mattresses (Fig. 1a) and bedding (Fig. 1b) than their non-HDM atopic siblings, there was wide variation in the levels of this allergen (also seen Fig. 3a,b). However previous studies suggest a threshold concentration of greater than 2 µg/g of *Der p I* to be regarded as high risk, and 10 µg/g as very high risk, for HDM sensitization [9,12,14,15,17]. Our data suggests that the threshold exposure level (absolute or concentration) resulting in sensitization may be significantly different between atopic children. In Fig. 1a six of the 20 non-HDM atopic children were exposed to concentrations above both these threshold levels but failed to be sensitized. Thus the concept of 'high risk' concentration levels for producing IgE in atopic patients may be unreliable. Studies by Marsh *et al.* suggest that genetic loci in the HLA region (chromosome 6) may contribute to an individual's risk of developing specific IgE responses to inhaled rag-weed allergens [30]. These loci may be important in determining the threshold exposure dosage at which an atopic becomes sensitized. Our data set is currently the subject of HLA-DR typing by dot blot hybridization with sequence specific oligoprobes to investigate this hypothesis.

We conclude that the predisposition to atopy in our families is primarily under genetic control at chromosome 11q [5,6] but that specific IgE response to HDM in the atopic children is, at least in part, determined by the magnitude of allergen exposure. Other factors (such as HLA loci) may influence this response. Our finding that the mattress and bedding are the most important sources of *Der p I* is consistent with acarological studies which have shown that the bed provides an ideal environment for mite growth due to its high humidity and abundance

of skin scales (the food source of mites) [31]. Studies of Papua New Guinean villages showed that introduction of blankets resulted in higher levels of mites and a corresponding increase in the prevalence of both mite sensitization and asthma [8]. A study of migrant Tokelauan children in New Zealand reported that their prevalence of asthma and other atopic disorders was higher than in children still resident in the Tokelau Islands but no different from Tokelauan children born in New Zealand [32]. Our observations may be relevant to the current concerns that the prevalence of the clinical manifestations of atopy are increasing and that this may be linked to increasing HDM levels and therefore exposure, caused by changes in domestic dwellings which promote mite growth [12]. By confirming both genetic predisposition to atopy and differences in allergen exposure between children within families, we have established that the environment plays an important role in causing HDM sensitization. This provides a useful and powerful model for examining the factors underlying the pathogenesis of atopic disorders.

Acknowledgments

We wish to thank the parents and children who participated in the study, Mrs Janet Evans and Mrs June White for technical assistance, Dr Jennifer Faux for advice in interpretation of IgE assays and Mr Colin Taylor, Medivac Ltd for supplying the Medivac vacuum cleaner. R. P. Young was funded by a Commonwealth Medical Scholarship and B. J. Hart was supported during this study by the National Asthma Campaign.

References

- 1 Pepys J. Atopy. In: Gell PGH, Coombs RRA, Lachmann PJ, eds. *Clinical Aspects of Immunology*. Oxford: Blackwell Scientific Publications, 1975:877-902.
- 2 Pruzansky JJ. Immunological and cellular aspects of immediate hypersensitivity. In: Patterson R, ed. *Allergic diseases—Diagnosis and Management*. Philadelphia: J.B. Lippincott, 1972:56.
- 3 Platts-Mills TAE. The biological role of allergy. In: Lessof MH, Lee TH, Kemeny DM, eds. *Allergy: An International Textbook*. Chichester: John Wiley & Sons, 1987:1-35.
- 4 Hopp RJ, Bewtra AK, Watt GD, Nair NM, Townley RG. Genetic analysis of allergic disease in twins. *J Allergy Clin Immunol* 1984; 73:265-70.
- 5 Cookson WOCM, Faux JA, Sharp PA, Hopkin JM. Linkage between Immunoglobulin E responses underlying asthma and rhinitis and chromosome 11q. *Lancet* 1989; i:1292-5.
- 6 Young RP, Lynch JR, Cookson WOCM, Hopkin JM. Confirmation of genetic linkage to atopy on chromosome 11 in sixty-four nuclear families and estimation of heterogeneity (abs). *Clin Sci* 1990; 79 (Sup 23):19p.

- 7 Rowntree S, Cogswell JJ, Platts-Mills TAE, Mitchell EB. Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. *Arch Dis Child* 1985; 60:727-35.
- 8 Turner KJ, Stewart GA, Woolcock AJ, Green W, Alpers MP. Relationship between mite densities and the prevalence of asthma: comparative studies in two populations in the Eastern Highlands of Papua New Guinea. *Clin Allergy* 1988; 18:331-40.
- 9 Smith TF, Kelly LB, Heymann PW, Wilkins SR, Platts-Mills TAE. Natural exposure and serum antibodies to house dust mite of mite allergic children with asthma in Atlanta. *J Allergy Clin Immunol* 1985; 76:782-8.
- 10 Walshaw MJ, Evans CC. The effect of seasonal and domestic factors on the distribution of *Euroglyphus maynei* in the homes of *Dermatophagoides pteronyssinus* allergic patients. *Clin Allergy* 1987; 17:7-14.
- 11 Price JA, Pollock I, Longbottom JL, Warner JO. Environmental factors influencing the development of allergy in asthmatic children—a retrospective investigation (abstract). Meeting of the ASCI. Cambridge 1988:s12/2.
- 12 Korsgaard J. Mite asthma and residency: a case-control study on the impact of exposure to house dust mites in dwellings. *Am Rev Resp Dis* 1983; 128:231-5.
- 13 Ishi A, Takaoka M, Ichinoe M, Kabasawa Y, Ouchi T. Mite fauna and fungal flora in house dust from homes of asthmatic children. *Allergy* 1979; 34:379-87.
- 14 Nordvall SL, Erickson M, Rylander E, Schwartz B. Sensitization of children in the Stockholm area to house dust mites. *Acta Paediatr Scand* 1988; 77:716-20.
- 15 Lau S, Falkenhorst G, Weber A, Werthmann I, Lind P, Buettner-Goetz P, Wahn U. High mite-allergen exposure increases the risk of sensitization in atopic children and young adults. *J Allergy Clin Immunol* 1989; 84:718-25.
- 16 Merrett TG, Burr ML, Butland BK, Merrett J, Miskelly FG, Vaughan-Williams E. Infant feeding and allergy: 12-month prospective study of 500 babies born into allergic families. *Ann Allergy* 1988; 6:13-20.
- 17 Sporik R, Holgate S, Platts-Mills TAE, Cogswell JJ. Exposure to house dust mite allergen (*Der p1*) and the development of asthma in childhood. *N Eng J Med* 1990; 323:502-7.
- 18 Price JA, Pollock I, Little SA, Longbottom JL, Warner JO. Measurements of airborne mite allergen in homes of asthmatic children. *Lancet* 1990; 336:895-7.
- 19 Platts-Mills TAE, Chapman MD. Dust mites: immunology, allergic disease and environmental control. *J Allergy Clin Immunol* 1987; 80:755-75.
- 20 Report of an International Workshop. Dust mite allergens and asthma—a worldwide problem. *J Allergy Clin Immunol* 1989; 83:416-27.
- 21 Kjellman N-IM. Predictive value of high IgE levels in children. *Acta Paediatr Scand* 1976; 65:465-71.
- 22 Bhalla RB. In: Benson E, ed. Advanced interpretation of clinical laboratory data. New York: Marcel Dekker, 1982:295-305.
- 23 Hart BJ, Fain A. A new technique for isolation of mites exploiting the difference in density between ethanol and saturated NaCl: qualitative and quantitative studies. *Acarologia* 1987; 28:251-4.
- 24 Hart BJ, Fain A. Morphological and biological studies of medically important house dust mites. *Acarologia* 1988; 29:285-95.
- 25 Fain A, Guérin B, Hart BJ. *Acariens et allergies*. Courtrai: Groeninghe Publ, 1988.
- 26 Luczynska CM, Arruda K, Platts-Mills TAE, Miller J, Lopez M, Chapman MD. A two site monoclonal antibody ELISA for the quantification of the major *Dermatophagoides* allergens *Der p1* and *Der f1*. *J Immunol Methods* 1989; 118:227-35.
- 27 Young RP, Hart BJ, Faux JA, Hopkin JM. House dust mite sensitivity: a comparison of immune response with exposure to four species of Pyroglyphidae. *Clin Exp Allergy* 1990; 20:319-25.
- 28 Swanson MC, Campbell AR, Klawns MJ, Reed CE. Correlations between levels of mite and cat allergens in settled and airborne dust. *J Allergy Clin Immunol* 1989; 83:776-83.
- 29 Kjellman N-IM. Predictive values of high IgE levels in children. *Acta Paediatr Scand* 1976; 65:465-71.
- 30 Marsh DG, Meyers DA, Bias WB. The epidemiology and genetics of atopic allergy. *N Eng J Med* 1981; 305:1551-9.
- 31 Bronswijk JEMH van. House dust biology for allergists, acarologists and mycologists. Zeist: Zoelmand, 1981.
- 32 Waite DA, Eyles EF, Tonkin SL, O'Donnell TV. Asthma prevalence in Tokelauan children in two environments. *Clin Allergy* 1980; 10:71-5.

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