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Sensitization to allergens of house-dust mite in adults with atopic dermatitis in a cold temperate region

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Background: An IgE-mediated contact reaction to airborne allergens has been suggested as one important pathogenetic mechanism in atopic dermatitis (AD). The house-dust mite (HDM) might be a common allergen involved. In Scandinavia, sensitization to HDM has been rare, probably because of the cold, dry climate. However, recent studies indicate high levels of domestic mites and HDM allergen in 15–20% of homes in central and northern Sweden.

Methods: To evaluate the importance of the HDM in patients with AD in the Stockholm region, we screened 81 adult Stockholm residents with AD, for the prevalence and degree of sensitization to the HDM, according to specific IgE (RAST), skin prick test (SPT), and atopy patch test (APT). We also assessed the HDM exposure in their homes and correlated the results with clinical history, severity of the dermatitis, and type of residence during childhood and today.

Results: The sensitization rate to HDM was high (56% according to RAST, 24% according to SPT, and 47% according to APT), and 20% of the patients were exposed to HDM allergens in their beds. Mite exposure seemed to aggravate the dermatitis in highly sensitized patients.

Conclusions: The results indicate that we have to take the HDM into account when discussing aggravating factors in adult patients with AD in the Stockholm region.

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New data on the pathogenesis of atopic dermatitis (AD) suggest that an IgE-mediated contact reaction to airborne allergens is one of the mechanisms involved. Various studies indicate that the house-dust mite (HDM) might be a common allergen involved. IgE receptors, both the low-

affinity receptor $Fc_\epsilon RII$ and the high-affinity receptor $Fc_\epsilon RI$, as well as IgE molecules, have been identified on epidermal Langerhans' cells (LC) in patients with AD (1–3). HDM-specific T cells (mainly of the Th2 subclass) have been found in AD lesions and in nonlesional skin of patients with AD after the atopy patch test (APT) with HDM allergen (4–6). Some 60–90% of patients with AD have elevated levels of HDM-specific IgE in serum (7), and 20–85% of patients with AD have a positive patch test reaction to HDM antigen (8–14). The level of specific IgE to HDM in serum is positively correlated with the clinical score of the patients' dermatitis (15), and the clinical score is positively correlated with the levels of HDM in the environment (15, 16) of AD patients. The levels of HDM in homes of patients with moderate to severe AD are significantly higher than in homes of nonatopics or patients with psoriasis (15, 17).

In the northern regions of Scandinavia, infestation by HDM has been rare (18). This is probably due to low indoor humidity during the winter season. However, recent studies (19–22) report detectable levels of domestic mites in 35% and high levels in 15% of dust samples from homes in central and northern Sweden, and detectable HDM-allergen levels (>7 ng/g) in 89% and high levels (>2000 ng/g) in 21% of dust samples from homes in the Stockholm area. A peak value was found in a dust sample from a home situated only 140 km from the Arctic Circle (20). One reason may be changes in building techniques during recent decades in Scandinavia, with better insulated homes for energy conservation. These buildings, which are less well-ventilated, accumulate humidity, which promotes HDM growth. A direct relationship between the poor ventilation and mite sensitization/infestation has been found (21).

To evaluate the importance of the HDM in patients with adult (aged ≥ 18 years) AD in the Stockholm region, we decided to screen for the prevalence and degree of sensitization to *Dermatophagoides pteronyssinus* and *D. farinae* in this group, according to specific serum IgE concentration, skin prick test (SPT), and APT. We also wanted to assess the HDM exposure in homes of patients with AD and correlate the results with clinical history, severity of the dermatitis, and type of residence during childhood and today.

Material and methods

Subjects

Eighty-one consecutive patients with adult AD attending the outpatient clinic of Dermatology, Stockholm Söder

Table 1. Characteristics of AD patients and healthy nonatopic controls

	Patients n=81	Controls n=20
Male/female	27/54	3/17
Age, years, median (range)	29 (18–65)	34 (23–55)
AD duration, years, median (range)	25 (1–64)	0
Respiratory symptoms*	63 (78%)	0
Positive clinical history**	35 (43%)	0
SCORAD Index (0–102)***, median (range)	40 (0–79)	0
Total IgE (kU/l)****, median (range)	810 (2–13 900)	30 (2–120)

*Rhinitis and/or asthma.
 **In questionnaire, reported aggravated skin symptoms in contact with house dust.
 ***Consensus report of European Task Force on AD (24).
 ****Reference range: 1.3–263 kU/l for 5–20-year-old individuals and 1.6–122 kU/l for adults (Pharmacia CAP System IgE FEIA).

Hospital, from December 1993 until September 1994, were included (Table 1). Exclusion criteria were the use of systemic corticoids, immune modulators or suppressants, PUVA and UVA/UVB treatment, or systemic antihistamines. Twenty healthy nonatopics were included as controls (Table 1). The study was approved by the local ethics committee, and the patients and controls gave their informed consent.

Questionnaire

Together with a physician (the same for all participants), a questionnaire was completed with information on place of birth, type of residence during childhood and today (single-family home or apartment) and where in the house the bedrooms were situated, clinical history of aggravated skin symptoms (rash and/or intensified itch) in contact with house dust (staying in a dusty environment, house cleaning, etc.), other atopic manifestations (rhinoconjunctivitis and/or asthma), and duration and seasonal fluctuations of the dermatitis.

Collection of dust sample and assessment of HDM exposure

Dust samples from bed mattresses were collected for 2 min/m² by the patients and controls using their own vacuum cleaners. Sufficient dust for analysis was available from 89 homes (71 patients and 18 controls). A filter bag (Medeca AB, Uppsala, Sweden) was fitted into the inlet tube of the vacuum cleaner. The subjects were instructed not to vacuum their mattresses for 14 days before collection of dust samples. After collection, the filter bags were sealed in

Table 2. Concentrations of dust-mite allergens in dust samples from bed mattresses

Mite allergens ng/g dust*	Median (range)	No. of patients	Controls
		n=71	n=18
Der p 1+Der f 1 <55		57 (80%)	18 (100%)
Der p 1+Der f 1 ≥55–<2000	96 (57–1800)	8 (11%)	0
Der p 1+Der f 1 ≥2000	77 500 (17 200–325 400)	6 (9%)	0
		n=14	
Der p 1 ≥55	4600 (86–38 400)	7 (50%)	
Der f 1 ≥55	21 100 (59–287 000)	10 (71%)	
Der p 1 alone ≥55	8700 (86–17 200)	2 (14%)	
Der f 1 alone ≥55	470 (59–59 600)	5 (36%)	

*Dust samples were collected by vacuuming mattresses for 2 min/m². Der p 1 and Der f 1 were measured with monoclonal antibody-based ELISA with lower detection limit of 55 ng/g of dust.

plastic bags and stored at –18°C until extracted. Each sample was weighed, and 100 mg of dust was extracted by rotation overnight at 4°C with 2 ml of borate-buffered saline, pH 7.4. After centrifugation, the extracts were stored at –20°C until assayed for allergen content. Allergen content for group 1 *Dermatophagoides* allergens (Der p 1 and Der f 1) was measured with a monoclonal antibody-based ELISA (kindly provided by Professor M. Chapman, Indoor Biotechnologies, Chester, UK). References with known amounts of Der p 1 and Der f 1 (UVA 87/03 and 87/02;93/02, respectively) were used, and results were expressed as nanograms per gram of dust. All samples were tested in duplicate. The coefficients of variation for Der p 1 and Der f 1 were below 6% and 16%, respectively. The lower detection limit for the allergens was 55 ng/g of dust.

Skin prick test (SPT)

The SPT was performed with commercially available extracts from ALK (Copenhagen, Denmark) with the potency of 10 HEP, which, according to the company, is equal to 20 000 BU/ml. The allergens tested were *D. pteronyssinus* and *D. farinae*, *Cladosporium*, *Alternaria*, birch pollen, hen's egg, cow's milk, and codfish. Test procedures were performed, according to Aas & Belin (23), on the volar part of the forearm on intact, nonsteroid-treated skin. The skin reactions were read after 15 min, and the wheal sizes were compared with positive histamine (histamine chloride 10 mg/ml) and negative control. A wheal size equal to the histamine reaction was scored 3+ in a scoring system of 1–5. A SPT reaction of ≥2+ was considered

positive. The same person, a trained nurse, performed all the tests.

Total and specific IgE

Total IgE and specific IgE concentrations in serum were determined with the Pharmacia CAP System, IgE and RAST, FEIA (Pharmacia and Upjohn AB Diagnostics, Uppsala, Sweden). IgE was measured against *D. pteronyssinus* and *D. farinae* as well as a broadleaf mix (alder, birch, hazel, oak, and willow), a mold mix (*Penicillium*, *Cladosporium*, *Aspergillus*, and *Alternaria*), a food mix (hen's egg, cow's milk, fish, wheat, peanut, and soybean), and the yeast *Pityrosporum orbiculare* (also denoted *P. ovale* or *Malassezia furfur*). The cutoff for total serum IgE was 2 kU/l and for RAST 0.35 kU/l. The RAST values were expressed in classes 0–6. A RAST class of ≥1 was recognized as positive.

Atopy patch test (APT)

Freeze-dried extract of *D. pteronyssinus* from ALK, normally used for immunotherapy, was mixed in two different vehicles; phosphate-buffered saline solution (the dilution fluid used in immunotherapy) and white petrolatum, and in two concentrations; 20 000 BU and 100 000 BU per ml of solution or gram of petrolatum. Volumes of 0.03 ml of solution preparations and 0.03/g of petrolatum preparations were applied in Finn chambers (8 mm) on tape (adhesive capacity=8 N/25 mm) stripped (×15) skin and clinically normal skin on the back for 24 or 48 h. Two control patches with the vehicles alone were added on both tape-stripped and intact skin. The series were read at 24 and 48 h in half of

the patients, and at 48 and 72 h in the other half. Scoring was performed by the standard reading procedure; -: no reaction, +: erythema, ++: erythema and induration/papules, +++: vesicles/exsudation. An APT reaction of $\geq++$ was recognized as positive. The same physician evaluated all tests.

Severity scoring of AD

The patient's clinical score was estimated according to the consensus report of the European Task Force on AD – the SCORAD Index (24). This index is based on a mathematical formula where extent ("rule of nine") and subjective symptoms account for 20% each of the total score, and intensity items (erythema, edema/papules, oozing/crusts, excoriations, lichenification, and dryness) represent 60%.

Statistical analysis

All variables are presented by descriptive statistics. Associations between different test methods (RAST, SPT, and APT) were analyzed by the Spearman rank correlation test (nonparametric), and comparative analysis by the Kruskal-Wallis nonparametric test. Comparison of categorical data was made with the chi-square test. *P* values of <0.05 were considered statistically significant.

Results

Exposure to HDM

Mite allergens (Der p 1, Der f 1, or both) were detected in 20% of the dust samples obtained from the patients' mattresses, compared with none from those of the nonatopic controls (Table 2). Der f 1 was the most common mite allergen. Nine percent of the samples from the patients contained very high levels of mite allergens, median 77 500 (17 200–325 400) ng/g dust, which is well above the level of 2000 ng/g proposed as a risk level for sensitization (25). Eleven percent had low levels, median 96 (57–1800) ng/g (Table 2).

Sensitization to HDM

A positive RAST to *D. pteronyssinus* and *D. farinae* was noted in 56% of the patients and 24% had a positive SPT to those allergens (Table 3). There was no difference between sensitization to *D. pteronyssinus* and that to *D. farinae*. Forty-seven percent of the patients had a positive APT

(Table 3). There was no correlation between the RAST and APT reactions ($r=0.19$, $P=0.09$), but there was a tendency to higher probability for a positive APT with higher RAST classes (4–6); 7/8 patients with RAST classes 4–6 had a positive APT while 15/37 with RAST classes 1–3 and 15/36 with a negative RAST had a positive APT (Table 4). There was a significant correlation between SPT and APT ($r=0.32$, $P=0.04$); 14/19 patients with a positive SPT also had a positive APT, while 23/62 with a negative SPT had a positive APT (Table 5).

Ten percent of the controls had specific IgE antibodies to *D. pteronyssinus* (RAST class 2). The same controls also had positive APT. Mite allergens were not detected in their dust samples. None of the controls had a positive SPT to mite allergens.

The prevalence of sensitization to HDM, according to RAST, SPT, and APT, in relation to exposure to mite allergens is presented in Table 6. There was a dose response relationship between the SPT and HDM exposure ($P=0.006$). A total of 16% of the patients with exposure levels below the detection limit for mite allergens had a positive SPT, compared to 37% of patients exposed to 55–2000 ng/g and 83% to ≥ 2000 ng/g. Patients exposed to low levels of mite allergen did not differ from the group with no detectable levels in sensitization, as measured by RAST. However, all six patients whose mite concentrations exceeded 2000 ng/g dust had a positive RAST. Forty percent of the patients without detectable levels of mite allergen in their mattresses had a positive APT, while 62% of those who were exposed to 55–2000 ng/g and 67% exposed to ≥ 2000 ng/g had a positive APT.

Respiratory symptoms (rhinitis and/or asthma) were significantly more frequent in patients sensitized to HDM than in nonsensitized patients. Of the patients shown by APT to be mite sensitized, 83% had respiratory symptoms; among those who did not show sensitization in APT, the figure was 55% ($\chi^2=7.23$; $P=0.007$). Correspondingly, among patients shown by RAST to be sensitized, 91% had respiratory symptoms, whereas 69% of those with a negative RAST had such symptoms ($\chi^2=6.10$; $P=0.01$). Among patients with positive SPT, 94% had respiratory symptoms compared to 77% with a negative test, but the difference was not statistically significant.

Sensitization to other allergens

About 70% of the patients were sensitized to broadleaf pollen, 50% to molds, and 45% to food allergens (Table 3). Most of the reactions were weak (RAST class 1 and SPT of

Table 3. Sensitization to house-dust mite and other allergens

RAST (classes)	0	1	2	3	4	5–6
<i>Dpt</i> * (n=79)	39 (49%)	8 (10%)	15 (19%)	10 (13%)	4 (5%)	3 (4%)
<i>Df</i> * (n=74)	33 (45%)	6 (8%)	19 (26%)	10 (13%)	4 (5%)	2 (3%)
<i>Dpt+Df</i> ** (n=81)	36 (44%)	8 (10%)	19 (24%)	10 (12%)	5 (6%)	3 (4%)
Broadleaf mix ***(n=73)	21 (29%)	51 (70%)	1 (1%)	0	0	0
Mold mix**** (n=75)	38 (51%)	37 (49%)	0	0	0	0
Food mix***** (n=77)	42 (55%)	34 (44%)	0	1 (1%)	0	0
<i>P. orbiculare</i> (n=74)	30 (40%)	6 (8%)	12 (16%)	20 (27%)	6 (8%)	0
Skin prick test n=81	0	1+	2+	3+	4+	5+
<i>Dpt</i> *	56 (69%)	7 (9%)	10 (12%)	7 (9%)	1 (1%)	0
<i>Df</i> *	59 (73%)	7 (10%)	9 (11%)	5 (6%)	0 (0%)	0
<i>Dpt+Df</i>	53 (65%)	9 (11%)	11 (14%)	7 (9%)	1 (1%)	0
Birch pollen	24 (30%)	8 (10%)	20 (24%)	25 (31%)	3 (4%)	1 (1%)
Mold	42 (52%)	24 (30%)	12 (14%)	3 (4%)	0	0
Food (milk, hen's egg, and fish)	43 (53%)	21 (26%)	7 (9%)	6 (7%)	1 (1%)	3 (4%)
Atopy patch test n=81	0	1+	2+	3+		
HDM extract*****	27 (33%)	17 (21%)	32 (40%)	5 (6%)		

**Dpt*: *Dermatophagoides pteronyssinus*, *Df*: *D. farinae*.
***Dpt+Df*: Mean RAST value of *Dpt* and *Df*.
***Broadleaf mix: alder, birch, hazel, oak, and willow.
****Mold mix: *Penicillium*, *Cladosporium*, *Aspergillus*, and *Alternaria*.
*****Food mix: egg, milk, fish, wheat, peanut, and soybean.
*****HDM extract in APT: freeze-dried HDM extract of *Dpt* from ALK, normally used for immunotherapy.
RAST class of ≥ 1 , SPT of $\geq 2+$, and APT of $\geq 2+$ were recognized as positive.

$\geq 2+$). Ten percent of the controls had positive SPT to birch pollen but were negative to the other allergens tested. A positive RAST to *P. orbiculare* was found in 59% of the patients, and the IgE levels were higher than to the other allergens tested.

The severity score

The severity score, according to the SCORAD Index, was correlated neither to the total IgE nor to specific IgE against *D. pteronyssinus* and *D. farinae*, except for two patients having extremely high levels (RAST class 6). There was no correlation with the SPT. Patients with strong APT reactions (+++) had a higher SCORAD Index, mean 51 (37–76), than those having negative (mean 42) or ++ (mean 42) reactions. The severity score seemed not to be related to mite exposure alone, but for highly sensitized patients,

according to RAST, the mite exposure seemed to increase the severity of the dermatitis (mean SCORAD Index=50, $n=4$), compared to nonexposed highly sensitized patients (mean SCORAD Index=25, $n=3$).

The clinical history

The clinical history of aggravated skin symptoms in contact with house dust was correlated neither with a positive RAST nor with a positive SPT against *D. pteronyssinus* and *D. farinae*. However, 21/35 patients (60%) who had a positive clinical history also had a positive APT.

Type of residence

Sixty-five percent of the patients were brought up in single-family homes. Of these patients, 63% had their bedroom on

Table 4. Correlation between serum IgE (RAST classes) to *Dermatophagoides pteronyssinus* and *D. farinae* and atopy patch test reactions to HDM extract

RAST (classes)	0	1–3	4–6	Total no. of patients
Atopy patch test				
0–1+	21	22	1	44
2+	15	12	5	32
3+	0	3	2	5
Total no. of patients	36	37	8	81
RAST class of ≥ 1 and APT of $\geq 2+$ were recognized as positive.				

the bottom floor. The rest were brought up in apartment houses; of these patients, 24% had their apartment on the ground floor. No difference was seen in sensitization to mites in the different groups.

Today, most of the patients (60%) live in apartments, and are to a lesser degree sensitized to mites than are the patients living in single-family homes (50% vs 64%, according to RAST). Patients living on the upper floor in apartment houses had the lowest sensitization rate (46%). The mite content in the patients' mattresses was higher in the single-family homes, but, on the other hand, the frequency of mite-containing mattresses was higher in apartments, although the concentrations were mostly <500 ng/g dust.

Discussion

Our study confirms the relatively high prevalence of mite allergens in central Sweden, as shown in recent epidemiologic studies (19–22, 26). The prevalence was higher in homes of patients with AD than in the homes of nonatopic individuals. This could be due to an AD-provoking influence of the mite allergen itself and/or a more mite-promoting environment in homes of AD patients. We found that

Table 5. Correlation between skin prick test to *Dermatophagoides pteronyssinus* and *D. farinae* and atopy patch test reactions to HDM extract

Skin prick test	0–1+	2–3+	4–5+	Total no. of patients
Atopy patch test				
0–1+	39	5	0	44
2+	21	11	0	32
3+	2	2	1	5
Total no. of patients	62	18	1	81
SPT of $\geq 2+$ and APT of $\geq 2+$ were recognized as positive.				

Table 6. Prevalence of sensitization to *Dermatophagoides pteronyssinus* and/or *D. farinae* in relation to exposure to mite allergens (Der p 1+Der f 1) in bed

Mite allergens (ng/g dust)	No. of patients exposed	No. of patients sensitized to mites		
		SPT	RAST	APT
<55	57	9 (16%)	32 (56%)	23 (40%)
≥ 55 –<2000	8	3 (37%)	4 (50%)	5 (62%)
≥ 2000	6	5 (83%)	6 (100%)	4 (67%)
RAST class of ≥ 1 , SPT of $\geq 2+$, and APT of $\geq 2+$ were recognized as positive.				

D. farinae was the main mite species in dust samples from mattresses, conflicting with the results of another Swedish study (22) that found an equal prevalence of *D. farinae* and *D. pteronyssinus* in the Linköping area in central Sweden. Wickman et al. (19–21) reported that *D. microceras* was the main source of mite allergen in the Stockholm area.

In 9% of the patients' homes, we found very high mite-allergen levels, but in most cases the levels were well below the proposed risk level for sensitization (2000 ng/g dust) (25, 27). The high sensitization rate among the patients (56% according to RAST) indicates that lower levels are of importance for sensitization. This agrees with other recent studies (26, 28). Although we found *D. farinae* to be the most frequent species, sensitization to *D. pteronyssinus* and *D. farinae* were equal, a finding which could be due to a high degree of cross-reactivity between the two HDM species (25).

The sensitization rate measured with RAST and APT was almost equal (56% and 47%, respectively), but the correlation between the test methods was low. This is not surprising, since the methods measure different events in the immune system. The RAST and SPT reflect immediate-type hypersensitivity, while the mechanism behind the APT reaction is considered to be a cell-mediated reaction via the LC in the epidermis. Several studies on the immunologic mechanism behind the APT reaction have been published in recent years (1–6, 29), indicating that allergen-specific IgE, through binding to IgE receptors on epidermal LC, can initiate a specific T-cell response (predominance of Th₂). The liberated cytokines then mediate the inflammatory response and IgE synthesis by the B cells. In our study, we found that a high RAST class (4–6) was associated with a higher probability of a positive APT. This indicates a potentiating ability of the IgE in the reaction. However, the presence of IgE seems not to be obligatory for the APT reaction, as it can take place in individuals without detectable allergen-specific IgE in serum, as shown in our

study as well as in others (14, 30–31). Further studies are needed to elucidate the mechanism behind the APT reaction. Another explanation of the low concordance between the RAST and APT may be that different allergen raw materials, with a varying amount of allergen content, are used in the two methods. In the SPT and the APT, the same *D. pteronyssinus* extract was used. This may explain why we found a significant correlation between APT and SPT, but not between APT and RAST. Only the APT was related to the clinical history of aggravated skin symptoms in contact with house dust, a finding which may be due to the fact that APT better reflects the immunologic reaction in AD. A strong APT reaction, as well as a high total serum IgE and high concentration of specific IgE, indicates more severe dermatitis. In this study, the severity score of the dermatitis was not correlated to mite exposure alone, but for highly sensitized patients, mite exposure seemed to aggravate the dermatitis.

In conclusion, this study confirms that the sensitization rate to HDM, measured with RAST, SPT, and APT, was high among adult patients with AD in the Stockholm region. Twenty percent of these patients had detectable levels of mite allergen in their beds. In 9%, the levels were very high,

but in the rest they were low. At present, there are no established risk levels for sensitization or developing clinical symptoms in AD. We found that the severity of the dermatitis seemed to increase by mite exposure in highly sensitized patients. Our study indicates that one has to take the HDM allergens into account when discussing aggravating factors in adult patients with AD in the Stockholm region. The diagnostic laboratory tools in AD are still crude. The APT is a promising method, but further standardization of the test is needed before it comes into clinical practice.

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