

Sensitization to storage mites in house dust mite (*Dermatophagoides pteronyssinus*) allergic patients. Comparison of a rural and an urban population

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Summary

In this study, sera collected from 50 patients (24 females, 26 males) with Type I allergy to house dust mite (*Dermatophagoides pteronyssinus*) were investigated for IgE antibodies specific for eight different mite species including storage mites of the families *Pyroglyphidae*, *Glycyphagidae* and *Acaridae*. According to their environment the patients were divided into two groups. Group I consisted of 24 (11 women, 13 men) farmers working and living in rural regions of Austria (Styria, Lower Austria), group II included 26 citizens of Vienna (13 women, 13 men). As expected, RAST investigations revealed a higher rate of sensitization to storage mites in the farmer group. Comparing the two patient groups, sensitization to *Lepidoglyphus destructor* and *Tyrophagus putrescentiae* was markedly increased in the farmer group. However, the sensitization rate to storage mites was also considerably high in city dwellers. Elevated levels of IgE specific for *Euroglyphus maynei* were more frequently observed in the urban collective. RAST-inhibition experiments suggest a partial crossreactivity between house dust mites and storage mites. In their living environment, patients with perennial Type I allergy are exposed to multiple different mite-derived allergens in addition to the well-known house dust mite allergens. These allergens lead to sensitization and are therefore of clinical importance.

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Introduction

The importance of house dust mites (HDM)—*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*—as cause of perennial, allergic symptoms of the airways is well established [1,2]. During the last years the major allergens of the pyroglyphid mites have been characterized [3,4], and it could be demonstrated, that the faecal balls represent the most important source of the mite allergens [5]. It could be shown in several studies that there exists a clear relationship between the amount of HDM-allergen present in the environment on the one hand and the severity of allergic symptoms in HDM-allergic patients on the other hand [6–8]. Also in patients with atopic dermatitis, a sensitization to HDM seems to be of pathogenetic importance [9,10].

Beside these pyroglyphid mites (comprising *Euroglyphus maynei*), a great number of other mites are known. They can be found in big numbers in granaries, grain storage facilities and barns [11]. These mites, commonly summarized as 'storage mites' comprise a wide range of genera including: *Glycyphagus*, *Acarus*, *Tyrophagus*, *Lepidoglyphus* etc. Similar to HDM, they possess allergenic properties, and sensitization can lead to allergic symptoms. Especially farmers and workers who are occupationally exposed to grain dust are concerned [12–16], therefore the term 'barn allergy' has been created for this particular kind of sensitization [12]. However, storage mites also exist in dwelling spaces, especially when relative humidity is high and flour products or other food stuffs are available.

Until now the clinical importance of the different species of storage mites for rural and urban homes is not yet well established and also the information about putative crossreactivities—leading to cross-sensitization

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and cross-allergenicity—between the HDM and the storage mites is still a matter of controversy [17–24].

In the present study, a collective of HDM allergic patients living in a rural or urban environment were investigated for specific IgE to representative members of the mite genus and the reactivity patterns (i.e. sensitization to different mites) were compared.

Materials and methods

Patients

Seventy patients participated in this study. Fifty of the 70 patients were allergic to HDM, proven by case history (perennial allergic rhinitis/conjunctivitis/asthma bronchiale), positive skin-prick test and RAST (Pharmacia, Uppsala, Sweden), classes for Pharmacia antigen d1 were in all cases >3 (>3.5 PRU/ml). The patients were subdivided into two groups.

Group I: Farmers: 24 subjects (11 females, 13 males) working and living in rural regions of Austria (Styria, Lower Austria), and suffering from perennial respiratory symptoms, eight out of 24 suffering from symptoms restricted to the upper respiratory tract and/or conjunctivitis, 18 out of 24 (75%) suffered from symptoms in the lower airways and had already experienced asthma attacks. Total IgE levels were in most of the cases elevated, they ranged from 81 to >2000 kU/l (mean: 586 kU/l). As most of the patients of this group were recruited in a lung-rehabilitation centre for farmers, the asthma rate in these individuals is considerably high.

Group II: Citizens: 26 subjects (13 females, 13 males) with perennial respiratory symptoms, who grew up, and lived permanently in an urban environment in Vienna, Austria. In this group 12 patients could be diagnosed as asthmatic (46%), whereas 14 out of 26 suffered from rhinitis/rhinoconjunctivitis. Total IgE levels ranged from 13 to >2000 kU/l, mean: 399 kU/l.

Control group: Twenty individuals (10 women, 10 men) working and living in rural areas of Austria, with a negative case history concerning HDM, and negative HDM RAST results. Ten of these farmers (5 women, 5 men) had normal total IgE levels, in 10 cases (5/5) PRIST values were elevated (180– >2000 kU/l).

IgE determination

IgE was determined using the RAST system (Pharmacia, Uppsala, Sweden) according to the manufacturers instruction. In detail RAST discs d1: *D. pteronyssinus*, d2: *D. farinae*, d3: *D. microceras*, d70: *A. siro*, d71: *L.*

Table 1. Taxonomy of mites

Family	Genus	Species
Pyroglyphidae	<i>Dermatophagoidae</i>	<i>D. pteronyssinus</i>
		<i>D. farinae</i>
		<i>D. microceras</i>
	<i>Pyroglyphinae</i>	<i>Euroglyphus maynei</i>
Glycyphagidae	<i>Glycyphagus</i>	<i>Glycyphagus domesticus</i>
	<i>Lepidoglyphus</i>	<i>Lepidoglyphus destructor</i>
Acaridae	<i>Acarus</i>	<i>Acarus siro</i>
	<i>Tyrophagus</i>	<i>Tyrophagus putreus</i>

destructor, d72: *T. putreus*, d73: *G. domesticus* and d74: *E. maynei* were used. Taxonomy of the mite species investigated are listed in Table 1. Total IgE was measured using the PRIST system (Pharmacia, Uppsala, Sweden) according to the manufacturers instruction.

Statistical evaluation

Comparison of the two groups was performed according to RAST results: IgE levels <0.35 PRU/ml were considered negative, RAST-class 1,2 (0.35–3.5 PRU/ml): weakly positive, >3.5 PRU/ml: positive. Statistical evaluation of the comparison of the results was performed using the Chi-square test.

Inhibition experiments

For the performance of RAST-inhibition, 10 sera containing elevated levels of IgE specific for either the allergen *Lepidoglyphus destructor* (d71), *Tyrophagus putreus* (d72) or *Euroglyphus maynei* (d74) were pooled. Aliquots (200 μ l) of the three serum pools were preincubated with *Dermatophagoides pteronyssinus* allergen-coated RAST discs overnight. In detail 0, 1, 2 and 4 discs were used. The preincubation was performed in the same way using the allergen discs d71, d72 and d74. Thereafter the serum samples were collected. The probes preincubated with d1 (*D. pteronyssinus*) were then tested for reactivity with d71 (*L. destructor*), d72 (*T. putreus*) or d74 (*E. maynei*), respectively. Sera preincubated with d71, d72 and d74 were tested for IgE-binding to d1. As controls d1 preincubated serum pools were investigated for decreased d1-specific IgE, d71, d72 and d74 preincubated pools were tested for the respective allergens. Moreover, every experiment was controlled by preincubation of serum samples with a non-related allergen—guinea-pig dander (RAST e6; Pharmacia)—to exclude the possibility of a non-specific inhibition.

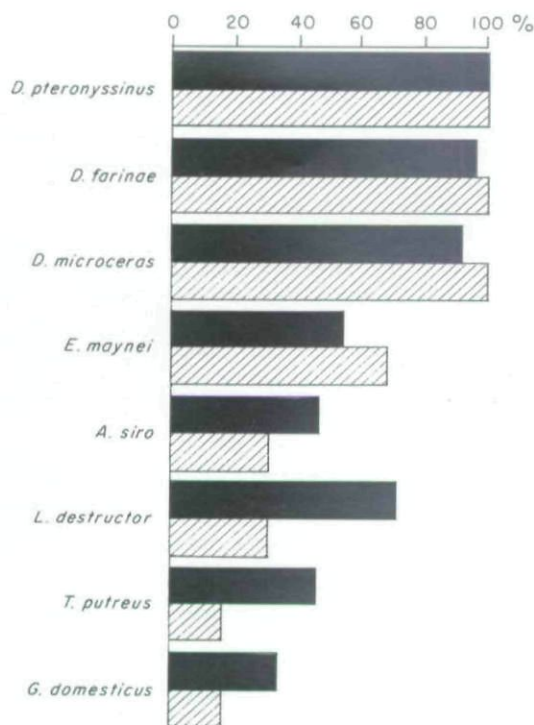


Fig. 1. Percentage of RAST-positive sera to different mite allergens. Comparison of ■, 24 farmers and ▨, 26 citizens.

Results

RAST results

Among the patients with established allergy to HDM, sensitization to storage mites was detected in 38 out of 50. Twenty-two out of 24 farmers (91.7%) had IgE which bound one or more storage mite allergens. Sixteen out of 26 city dwellers (61.5%) reacted with at least one of the storage mites. Regarding the storage mite RAST experiments, for group I 91 out of 120 (75.8%) RAST evaluations for storage mite were positive, in group II 61 out of 129 (47.3%), indicating, that sensitization to several storage mites was more frequently found in rural patients. Comparison of IgE-binding to allergens from different mites in farmers and citizens are summarized in Fig. 1.

In our non-allergic control group slightly elevated specific IgE concentrations were detected in two out of 20 subjects (2 out of 160 measurements). In one case there was a RAST class 1 (<0.7 PRU/ml) for antigen d71 and in the other case RAST class 2 for antigen d74. As expected, all the RAST measurements for house dust mite allergens were negative.

Statistical analysis of the distribution of sensitization in groups I and II

Comparison of the farmers with city dwellers shows that sensitization to storage mites in addition to HDM is more frequent in a rural than in an urban population. Statistical analysis showed, that in this respect especially sensitization to *L. destructor* and *T. putreus* is more frequent in farmers. Chi-square test analysis (taking the IgE levels into consideration) revealed significance for this association ($P < 0.005$ for *L. destructor*, $P < 0.01$ for *T. putreus*). On the contrary the HDM-allergic urban population displayed more frequently elevated specific IgE antibody levels to *E. maynei* (significance: $P < 0.05$).

Inhibition experiments

Preincubation of the three serum pools reactive for either *L. destructor* (d71), *T. putreus* (d72) or *E. maynei* (d74) with RAST paper discs coated with *D. pteronyssinus* allergens (d1), led in all cases to a dose dependent decrease of reactivity to the respective allergen. This effect was most pronounced using the *T. putreus* (d72)-reactive serum pool. However, preincubation of the serum pools with *L. destructor* (d71), *T. putreus* (d72) or *E. maynei* (d74) RAST discs revealed no blocking effect of d1 RAST in case of d72 and d74 and only a weak inhibition in case of *L. destructor* (d71).

Controls: Preincubation and subsequently testing for the same antigen led to strong inhibition in a dose dependent manner. Guinea-pig dander extract did not display any blocking ability. The results of the RAST inhibition experiments are summarized in Fig. 2.

Discussion

The so-called storage mites are organisms living in grain store rooms and other food magazines [11]. It is known that they possess allergenic properties, which can lead to Type I allergies in atopic individuals [12–16]. However, the frequency of sensitization to the different types of mites have not been sufficiently investigated yet and it is not clear, whether these sensitizations are due to cross-reactive IgE antibodies. In this study, a collective of 50 patients with clinical sensitization to HDM allergens was investigated for the presence of specific IgE for eight mite species belonging to the families *Pyroglyphidae*, *Glycyphagidae* and *Acaridae*. We wanted to know, whether the risk of storage mite allergy could be connected with the living environment of disposed (HDM-allergic) individuals. Therefore our patients were chosen in such a way that 24 farmers (living and working in rural regions of Austria) and 26 citizens, who grew up and lived perma-

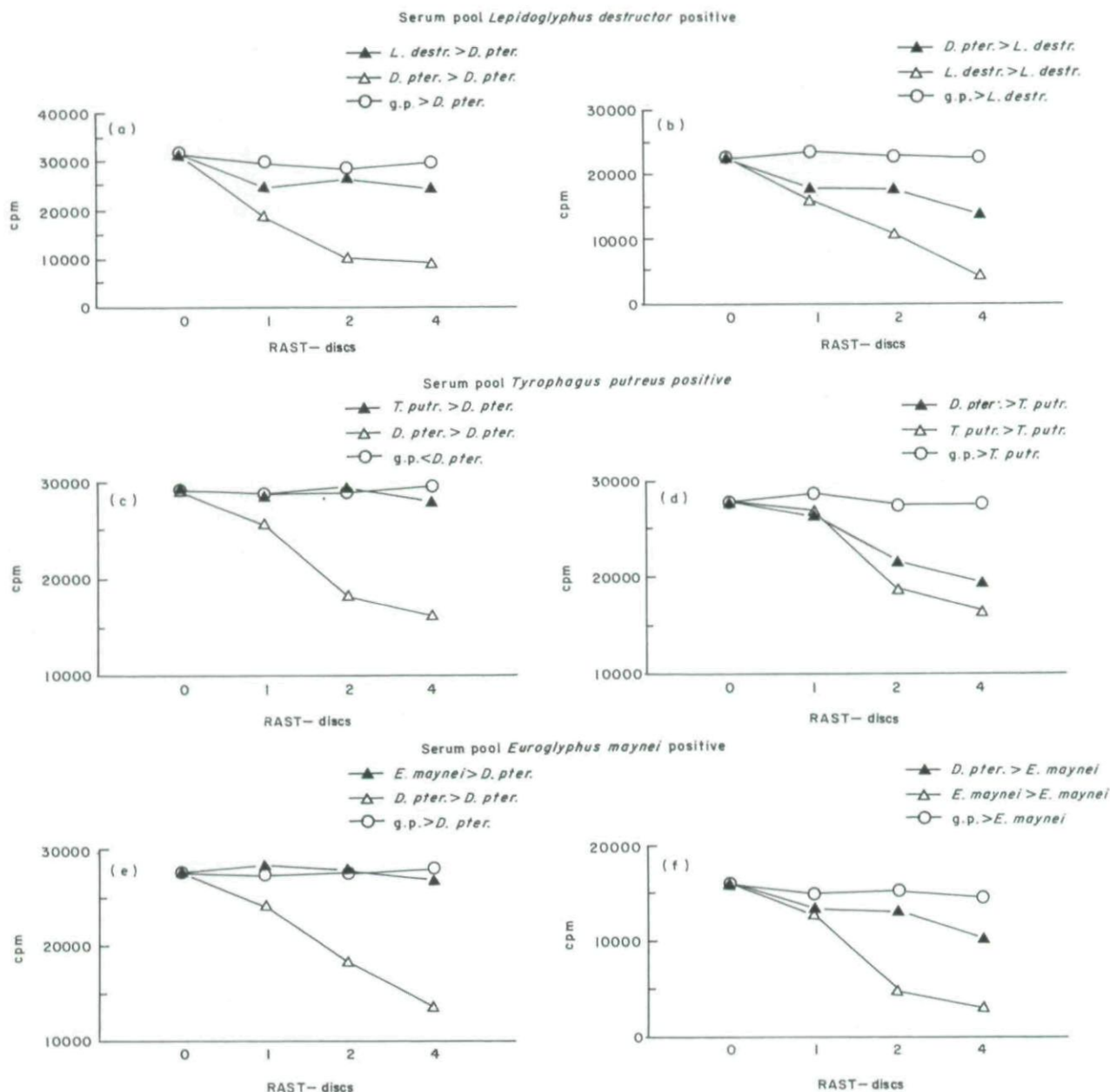


Fig. 2. RAST-inhibition. (a) Inhibition of *Dermatothoragoides pteronyssinus* RAST by preincubation of a *Lepidoglyphus destructor* RAST-positive serum pool with *D. pteronyssinus*, *L. destructor* and guinea-pig dander RAST discs. (b) Inhibition of *Lepidoglyphus destructor* RAST by preincubation of a *L. destructor* RAST-positive serum pool with *D. pteronyssinus*, *L. destructor* and guinea-pig dander RAST discs. (c) Inhibition of *D. pteronyssinus* RAST by preincubation of a *Tyrophagus putrescens* RAST-positive serum pool with *D. pteronyssinus*, *T. putrescens* and guinea-pig dander RAST discs. (d) Inhibition of *T. putrescens* RAST by preincubation of a *T. putrescens* RAST-positive serum pool with *D. pteronyssinus*, *T. putrescens* and guinea-pig dander RAST discs. (e) Inhibition of *D. pteronyssinus* RAST by preincubation of a *Euroglyphus maynei* RAST-positive serum pool with *D. pteronyssinus*, *E. maynei* and guinea-pig dander RAST discs. (f) Inhibition of *E. maynei* RAST by preincubation of a *E. maynei* RAST-positive serum pool with *D. pteronyssinus*, *E. maynei* and guinea-pig dander RAST discs.

nently in an urban environment, could be compared (Fig. 1). Indeed, we could detect a higher rate of sensitization to all storage mites in the farmers group (significant associations for *L. destructor* and *T. putres*), whereas sensitization to *E. maynei* was found in almost all patients in urban regions. Statistical analysis revealed significance for this association. *E. maynei* is a pyroglyphid mite which can be found as common as *D. pteronyssinus* in the homes of individuals with asthma [25].

The connection of particular sensitizations with the environment of our patients' groups led to the assumption, that in some cases the allergy to particular storage mites was maybe not due to cross-reacting IgE antibodies, but more to intensive exposure in the living area, leading to a parallel-sensitization. A recent study stated not to find any correlation between positive RAST to *D. pteronyssinus* and sensitization to any single storage mite investigated (*L. destructor*, *T. putrescentiae*, *G. domesticus*, *A. siro*; 21), in another publication no significant correlation could be detected between *D. pteronyssinus* and *L. destructor* or *E. maynei* [22]. However, according to Luczynska *et al.* [23] all individuals with a positive RAST to storage mites (*A. siro*, *L. destructor* or *T. longior*) had specific IgE to *D. pteronyssinus*. The discrepancy of the information available is probably due to the different test collectives in the studies published. All our patients were sensitized to HDM allergens, whereas in the above mentioned publications other inclusion criteria were chosen. Inhibition experiments using storage mite and HDM extracts were described in several publications and revealed a quite complex situation. Hage-Hampsten *et al.* [17] could not find any significant correlation between specific IgE levels to any storage mite and *D. pteronyssinus* using RAST inhibition, concluding that each of the storage mites possess their own unique allergens. However, later reports, using immunoblot or crossed radioimmuno-electrophoresis revealed distinct proteins with crossreactive properties for *D. pteronyssinus* and *L. destructor* [19] and for *D. pteronyssinus*, *L. destructor* and *A. siro* [20] respectively. In our experiments, using RAST inhibition, it was possible to inhibit the IgE reactivity to storage mite allergens by preincubation of sera with HDM extract, indicating the dominant role of this sensitization. However, it was not possible to markedly diminish IgE-binding to HDM allergens by depleting sera from specific IgE to *T. putres* or *E. maynei* before testing for HDM reactivity. Only *L. destructor* seemed to have a weak blocking capacity in this respect (Fig. 2). The results of our inhibition experiments suggest shared determinants within these allergen extracts, but provide also evidence for the existence of species-specific epitopes.

We consider the knowledge of sensitizations connected

with particular living environments as important. Especially the fact that sensitization to particular storage mites (e.g. *G. domesticus*, *A. siro*) is not only frequent in farmers, but also in patients living in an urban surrounding leading to parallel-sensitizations with non-crossreacting mite-derived allergens could improve diagnosis and maybe therapy of perennial allergic diseases. In our urban patients collective, 16 out of 26 city dwellers (61.5%) displayed considerable amounts of specific IgE to at least one storage mite. Therapeutical administration of *D. pteronyssinus* extracts might not be sufficient for inducing tolerance to other mite allergens in the living environment. Therefore, an additional sensitization to storage mites or other pyroglyphid mites should be considered as one reason for failure of specific immunotherapy of HDM-allergic patients with perennial symptoms. Tests using purified or recombinant mite allergens [26–28] will certainly elucidate, which of the proteins in the HDM extracts are involved in a putative crossreactivity with storage mite allergens.

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