

Inverse correlation of domestic exposure to *Dermatophagoides pteronyssinus* antigen patch test reactivity in patients with atopic dermatitis

C. GUTGESELL, A. SEUBERT, V. JUNGHANS and CH. NEUMANN

Department of Dermatology, Göttingen University, Göttingen, Germany

Summary

Background In recent years considerable interest in the pathogenetic role of aeroallergens exacerbating atopic dermatitis (AD) has emerged. The 'atopy patch test' with aeroallergens was introduced by Platts-Mills *et al.* as an experimental model and as a diagnostic tool. However, its relevance for the clinical manifestation of AD is still not clear.

Objective We asked whether there is a relationship between the individual antigen exposure to the major allergen of *Dermatophagoides pteronyssinus* (Der p 1) and the immunological markers of sensitization to Der p 1 or the clinical severity of AD.

Methods We investigated 92 patients with moderate to severe AD. For clinical evaluation the SCORAD severity score was used. Patch tests were performed with purified Der p 1. Specific IgE was measured by a commercial assay. Der p 1 exposure was quantified in a sample of the patient's mattress dust by using a commercial ELISA.

Results No correlation between SCORAD, Der p 1 exposure and RAST could be established. However, there was an unexpected significant inverse correlation between the quantity of mite antigen in the mattress dust and patch test reactivity. Patients with a high antigen load ($>25 \mu\text{g/g}$) mostly had a negative patch test. Also, when Der p 1 was correlated to the mattress area (m^2) in this group all patch tests were negative. A possible explanation could be that continuous exposure of the skin to house dust mite allergen Der p 1 may induce a down-regulation of the skin immune system of patients with AD.

Conclusion Although the mechanism of this phenomenon is presently unknown, our study shows that a positive allergen patch test alone should not be an indication to undertake allergen exclusion measures in AD patients.

Keywords: atopic dermatitis, atopy patch test tolerance, house dust mite, protein antigen

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Introduction

Sensitization to *Dermatophagoides pteronyssinus*, as evidenced by specific IgE, is a hallmark of atopy present in 40–80% of atopic patients [1,2]. Several reports quantifying the amount of Der p 1 antigen in the households of sensitized atopic individuals have demonstrated the existence of a positive correlation between Der p 1 exposure and mucosal symptoms of atopy such as allergic rhinoconjunctivitis and

asthma [3–5]. Furthermore, evidence for a pathogenetic role of Der p 1 for the development of asthma has been provided by house dust elimination studies, especially in children [6,7].

More recently studies have been conducted correlating quantitative amounts of Der p 1 in households with the incidence of atopic dermatitis (AD), the skin manifestation of atopy [8,9]. These studies were stimulated by the findings of Platts-Mills *et al.* and other investigators who showed that the application of Der p 1 antigen to the epidermis leads to a considerable percentage of positive patch test reactions in sensitized individuals with AD [10–12]. Moreover, there

Correspondence: Ch. Neumann, von-Siebold-Straße 3, D-37075 Göttingen, Germany.

is convincing evidence that allergen patch test sites, harbouring allergen-specific T cells at a higher frequency than the blood [13], represent a special type of late immunoreaction of the skin as shown by several authors including ourselves [14–17]. These findings suggest that a significant environmental Der p 1 load with high concentrations in the beds of atopic individuals, may exacerbate AD. In fact, a positive correlation between the individual's exposure to Der p 1 and his or her risk to develop AD has been demonstrated [18]. Thus, assuming that the allergen patch test is a useful model for the development of AD lesions, patients with a high individual exposure to Der p 1 in their households should react positive. It was the aim of this study to test this hypothesis by correlating the quantity of Der p 1 antigen in the patient's mattress with his individual patch test result and Der p 1-specific IgE in his serum. There was an unexpected inverse correlation between the exposure to Der p 1 and the ability to develop a late phase reaction to Der p 1.

Methods

Patients with moderate to severe AD aged 16–47 years (median age 24 years, 48 female, 44 male) were included in this study. Clinical severity of AD was evaluated by SCORAD [19] with a median score of 41. In addition to AD, 32 of the patients reported to suffer from asthma either at the time of testing or earlier in life.

Dermatophagoides pteronyssinus-specific serum-IgE was determined by the CAP-method (Pharmacia, Freiburg, Germany). Only patients with a RAST class ≥ 3 were included in this study. Patients had neither received systemic nor topical steroids for at least 4 weeks. Der p 1 patch tests were performed and evaluated as described before [14]. Briefly, purified Der p 1 as controlled by SDS-PAGE electrophoresis (Bencard, Smith Kline Beecham, Munich) was applied on uninvolved skin of the back and covered by large Finn Chambers on Scanpor (Hermal, Reinbek, Germany) for 24 h. Patch test results were read at 24, 48 and 72 h.

Patients with doubtful patch test reactions were excluded from this study. Dust samples were collected from the patients' mattresses as described by Lau *et al.* [20]. Standardization of house dust sampling was performed as published [21]. The dust was weighed, Der p 1 was extracted and quantified by ELISA (Scherax, Hamburg, Germany) according to the manufacturer's instructions. The measuring range for Der p 1 was 2–200 ng per mL extract. Samples above 200 ng/mL were further diluted with dilution buffer and subjected to another ELISA. The standard curve was not extrapolated beyond the lowest concentrations supplied by the manufacturer. Results were calculated as μg Der p 1 per gram mattress dust or per m^2 mattress area. For statistical analysis the Mann–Whitney U-test was applied.

Results

Correlation of Der p 1 antigen to quantity of mattress dust

To evaluate whether the amount of dust collected from the patient's mattress reflects the amount of Der p 1 antigen he or she is exposed to, both parameters were correlated. As shown in Fig. 1 there was no statistically significant correlation as there clearly was a significant number of individuals who collected more than 1 g of dust with no or little Der p 1 antigen in it. On the other hand, there were also some individuals with a low dust load on their mattress containing, however, a high concentration of Der p 1 antigen. These results demonstrate that measuring Der p 1 antigen and the overall dust load on the mattress more precisely reflects the individual allergen exposure than only measuring the amount of dust, as advocated by some authors [21].

Correlation of Der p 1 exposure to clinical parameters

The amount of Der p 1 antigen per gram mattress dust collected by each patient was correlated to individual clinical parameters. Figure 2 illustrates this for specific IgE in the serum. Figure 3 shows the result of the assessment of SCORAD either in relation to Der p 1 antigen per gram dust (Fig. 3a) or per m^2 of mattress area (Fig. 3b). Although there was a tendency towards a positive correlation in Fig. 3(b), this was not significant ($P = 0.055$).

Correlation of Der p 1 patch test to clinical parameters

A test site showing erythema and papules or erythema and vesicles after 48–72 h was regarded as a specific delayed type

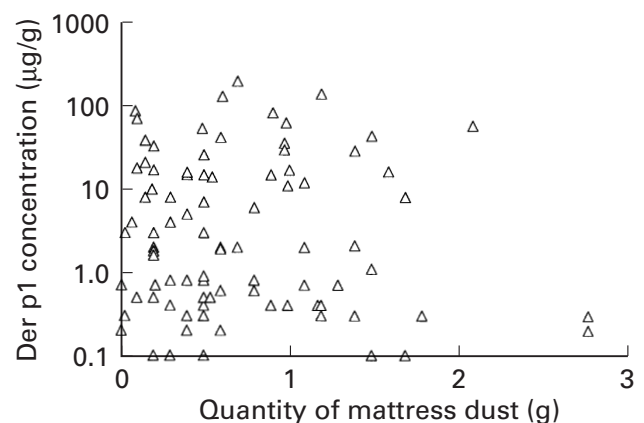


Fig. 1. Correlation of Der p 1 concentration to total amount of dust collected. Der p 1 exposure was quantified in dust samples collected from the patients' mattresses in a standardized manner. The dust was weighed and Der p 1 concentration in the dust was determined by a commercially available ELISA. No significant correlation was found.

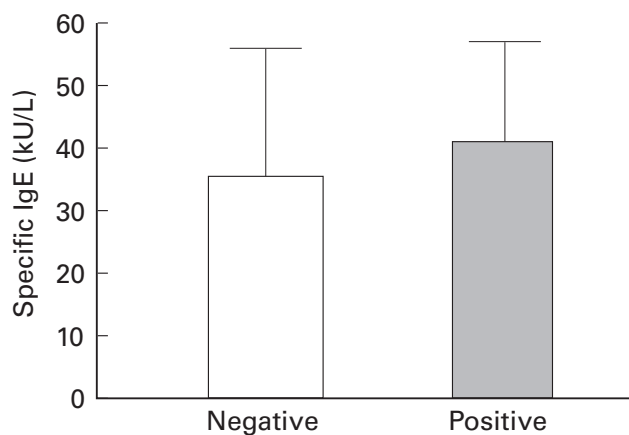


Fig. 2. Correlation of Der p 1 patch tests to specific IgE. The difference between specific IgE concentrations in the serum of patients with a positive and a negative Der p 1 patch test was not significant.

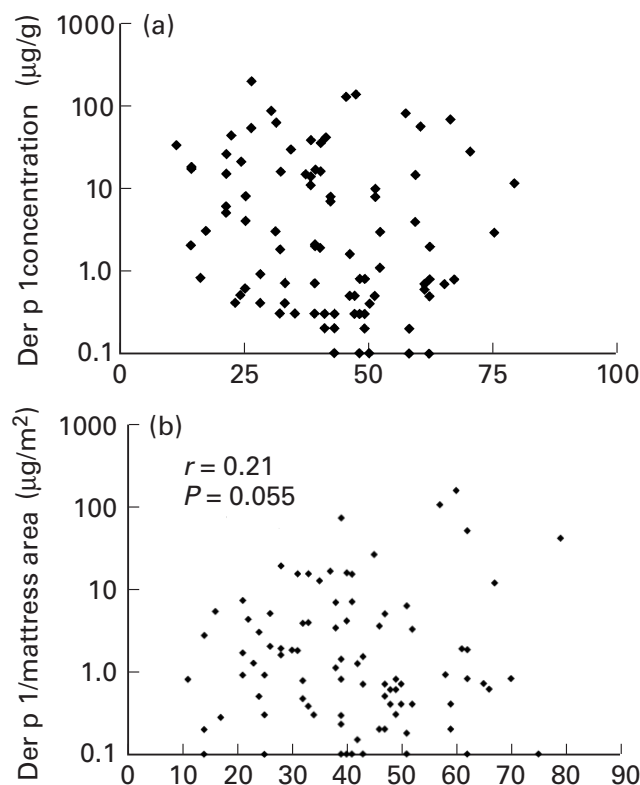


Fig. 3. Correlation of SCORAD to Der p 1 exposure. The atopic dermatitis severity score (SCORAD) is correlated to the individual house dust mite antigen exposure depicted as µg Der p 1 per gram mattress dust (a) or as µg Der p 1 per m² mattress area (b). There is no significant correlation ($P = 0.055$ in b).

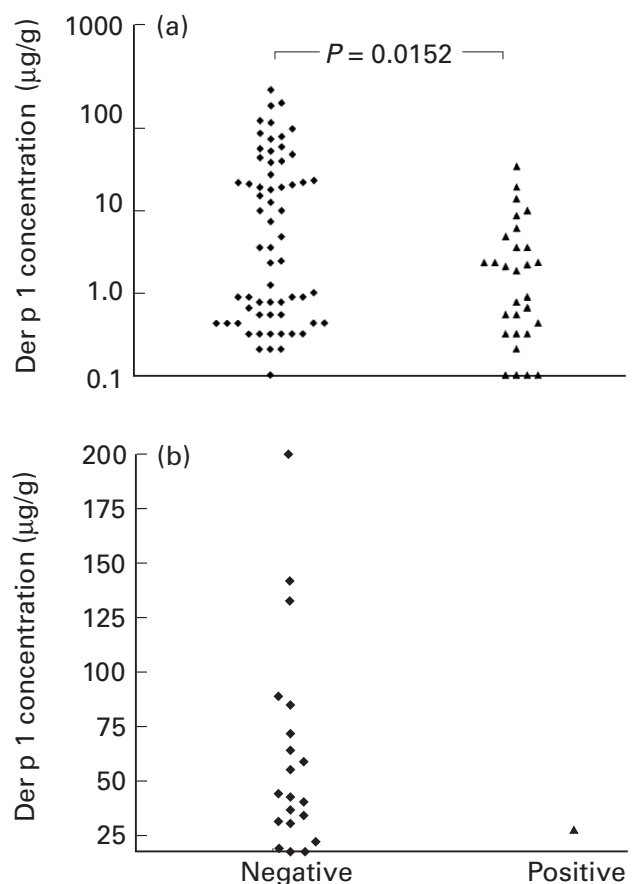


Fig. 4. Der p 1 per gram mattress dust in relation to Der p 1 patch test reactivity. (a) Patch tests were performed with Der p 1 as described; 63 patients showed a negative Der p 1 patch test; 29 reacted positively. Results are shown on a log scale. Patients with positive patch tests showed statistically significant lower amounts of Der p 1 per gram dust ($P = 0.015$, Mann–Whitney *U*-test). (b) Results of 21 patients with the highest Der p 1 load per gram dust are shown on a linear scale; only one had a positive patch test.

reaction to Der p 1 antigen. Neither the level of specific IgE in the serum, nor the SCORAD measured at the time of patch testing, nor the presence or absence of asthma showed a positive correlation with the outcome of the patch test (not shown).

Correlation of the Der p 1 patch test to Der p 1 exposure

In Fig. 4 the individual Der p 1 concentration in the patient's mattress dust is correlated to the outcome of the patch test reaction. In both patients groups $\approx 50\%$ of the dust samples contained less than $2 \mu\text{g}$ Der p 1 per gram dust (Fig. 4a). Interestingly, only the group with negative patch test results harboured a significant part of individuals with a high antigen exposure (Fig. 4a). While 16/63 individuals with a negative patch test had more than $25 \mu\text{g}$ Der p 1 per gram mattress dust, this was the case in only 1/29 individuals with

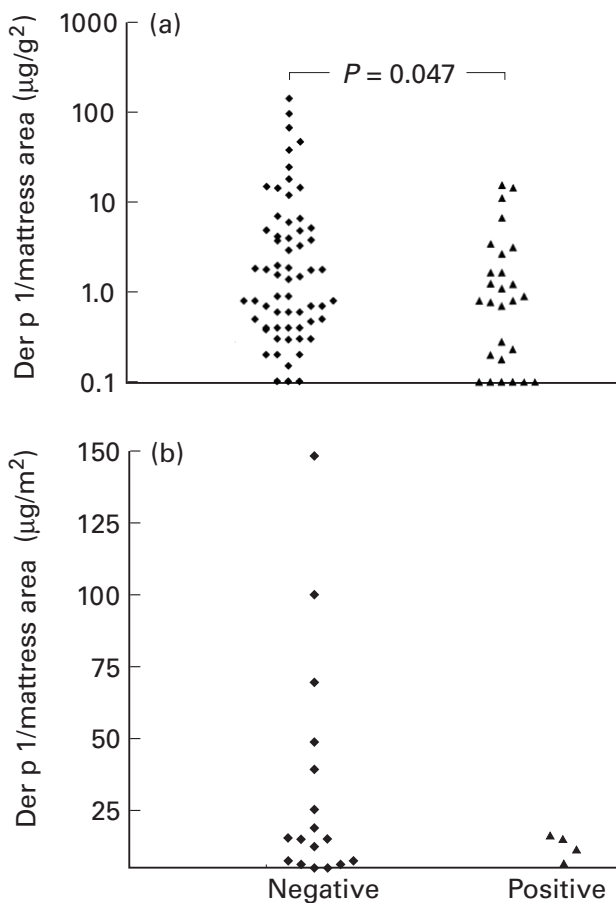


Fig. 5. Der p 1 per m² mattress area in relation to Der p 1 patch test reactivity. (a) Antigen load is expressed as Der p 1 per m² of mattress area; 63 patients showed a negative Der p 1 patch test; and 29 a positive patch test. Results are shown on a log scale. (b) Results of 21 patients with the highest load per m² are shown on a linear scale, only four had a positive patch test.

a positive patch test. This is shown on a linear scale in Fig. 4(b). The difference was statistically significant ($P < 0.05$) and was also obvious when the quantity of Der p 1 per square meter of mattress area was taken as a parameter for antigen exposure (Fig. 5a and b). Out of those 21 patients with the highest load of Der p 1 per gram dust only one had a positive patch test and patients with $> 25 \mu\text{g}$ Der p 1 per m² mattress area never had a positive patch test. Thus, positive allergen patch tests with Der p 1 are associated with relatively low antigen exposure. Moreover and possibly more importantly, it appears that an antigen load $> 25 \mu\text{g}$ per gram dust is usually associated with a negative patch test in the exposed individual.

Discussion

In recent years the allergen patch test has provoked considerable interest and its sensitivity, specificity and correlation

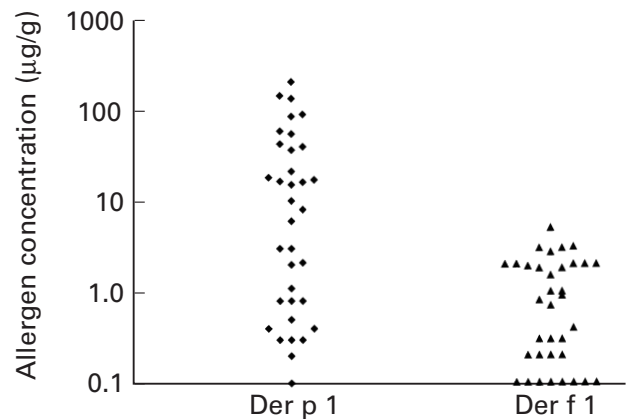


Fig. 6. Comparison of Der p 1 and Der f 1 exposure. Quantities of Der p 1 and Der f 1 are compared in the individual dust samples collected by 35 patients. Der f 1 never exceeded the amount of Der p 1.

with immediate-type reactions in individuals with AD have been repeatedly investigated [10–12]. Der p 1 has proved to be the allergen which yields highest positive patch test rates in patients with AD [12]. The specificity of this test has been further confirmed by the presence of lesional T cells reacting specifically to Der p 1 and displaying a distinct cytokine pattern [13–15,17].

Depending on the method used, ≈ 30 –70% of patients with AD who are sensitized to Der p 1 have been shown to develop this late type of immune reaction. Despite this fact its significance for the pathogenesis and individual course of the disease are presently unknown. Several authors including ourselves have proposed that AD might be regarded as a distinct type of contact allergy to aeroallergens [22]. In this case the atopy patch test with protein allergens of the house dust mite should serve as a model for AD similar to widely accepted patch testing with haptens in contact-sensitized individuals. It should be expected that in sensitized atopic individuals a positive atopy patch test indicates a high individual exposure to the allergen sustaining this special type of contact allergy to the protein antigen. Surprisingly, the present work reveals that this is not the case as we found a statistically significant inverse correlation between Der p 1 exposure and Der p 1 patch test reactivity in adult patients with AD.

For the following reasons it is unlikely that methodological errors account for this result: patch testing and sampling were performed in the same individual within 2 weeks. A second dust sampling and patch testing after 4 months performed in a small group of patients ($n = 5$) gave the same results and thus confirmed reproducibility (results not shown). *Dermatophagoides farinae* which might have been missed in the house dust by the ELISA test might have cross-reacted *in vivo* with the mite extract used for patch testing and might have yielded positive test results. To

exclude that *Dermatophagoides farinae* has contributed to the observed inverse correlation, dust of 35 mattresses was analysed in parallel for the *Dermatophagoides farinae* antigen (Der f 1) with a Der f 1-specific ELISA. Figure 6 shows that the level of Der f 1 was generally low, and it never exceeded the amount of Der p 1 found in the specimen which excludes an important role for Der f 1 in these patients.

Patients with the strongest sensitization may have cleared the dust from their homes resulting in lower Der p 1 levels at the time of patch testing. This possibility is, however, unlikely because only those patients were investigated who had not previously undertaken systematic allergen avoidance measures. Also, as evidenced by specific IgE levels, sensitization did not differ between the patch test-positive and -negative patients.

Allergen avoidance measures clearly have proved useful in house dust mite-sensitized patients with allergic asthma [1,2]. As others have shown that the concentration of Der p 1 in the mattress dust reflects the overall antigen exposure of the individual, it also seems to be a useful parameter in deciding on allergen exclusion measures. So far, only few studies on the quantification of Der p 1 exposure have been performed. Henderson *et al.* have not been able to demonstrate a positive correlation between Der p 1 exposure and severity of AD [8]. On the other hand a recent study suggests that strict avoidance of Der p 1 by encasing the mattress and cleaning the bedroom floor with acaricides may improve AD [9]. These results, however, need further confirmation. In the present study no correlation of the individual's SCORAD (measured at the time of dust sampling) with the outcome of his patch test could be established. This may be due to the fact that the evaluation of the SCORAD exclusively at one time point does not reflect the disease severity. Longitudinal observation of the individual course of the dermatitis have to be correlated with repeated Der p 1 patch testing to address this question. The results presented so far show that a single positive allergen patch test alone should not be an indication to undertake allergen exclusion measures in AD patients.

It is tempting to speculate that at least in some of the patients there is a causal relationship between the amount of Der p 1 in the patient's dust and the outcome of the patch test reaction. Approximately 2 µg Der p 1 per gram dust are regarded by many authors as a threshold for the induction of specific IgE in genetically predisposed individuals [23]. Most of our patch test-positive patients had a concentration of Der p 1 ranging from 0.1 to 10 µg per gram dust. Many patch test-negative patients were exposed to a 10–100-fold Der p 1 concentration above the sensitizing level. In fact only 1/17 patients with more than 25 µg Der p 1/g dust had a positive patch test. On the other hand, the amount of Der p 1 needed to elicit a positive patch test reaction lies above 1000

PNU per gram vehicle. This is ≈ 100-fold higher than the patient's domestic antigen exposure observed in his mattress dust and expressed as Der p 1 per gram dust or per m² mattress area. We therefore hypothesize that in some of the patients continuous exposure to amounts of allergen several fold above the sensitizing concentration induces down-regulation of reactivity against the experimental dose of allergen applied by patch testing.

Induction of anergy is a well-known phenomenon in the immunoregulation of contact hypersensitivity [for overview 24]; and several mechanisms may be responsible including down-regulation of molecules on antigen-presenting cells and the induction of TH2 cytokines. Although the mechanism of the observed inverse relationship of Der p 1 exposure and patch test results remains presently unknown, our data clearly show that the pathogenetic role of Der p 1 in the development of AD may be more complex than so far suggested.

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