

Major *Dermatophagoides* mite allergen, Der 1, in soft toys

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Summary

Background Allergen avoidance is recommended when treating atopic asthma.

Objectives Soft toys are often kept in close proximity to children and may serve as a source of exposure. Due to the potential danger from the mite allergen content of these toys, Der 1 from toys was measured.

Methods We quantified the level of Der 1 (Der p 1 + Der f 1) in both 30 new and 174 old soft toys (weight 216 ± 2.5 g, height 26.5 ± 3.4 cm), as well as in washed and in vacuumed soft toys. Dust was collected using an electric vacuum cleaner. Der 1 was measured by monoclonal antibody-based enzyme-linked immunosorbent assay (MoAb-ELISA).

Results In brand new toys Der 1 was $0.1 \mu\text{g/g}$ of dust, and in toys used for 1 year, 9.0; 2 year, 22.2; 3 year, 18.9; and more than 4 year, $22.7 \mu\text{g/g}$ of dust. Der 1 in new toys was measured every 4 months for 1 year. Der 1 rapidly increased 10- to 20-fold in the first 4 months, but there was no clear seasonal change. In toys washed using a chemical detergent or cleaned using a vacuum cleaner, there was a statistically significant ($P < 0.001$) decrease in Der 1 in the washed group, but not in the vacuumed group.

Conclusion These results confirm that mite allergens accumulate rapidly in toys to form a potentially important source of allergens and that washing toys with a chemical detergent is effective in the reduction of allergens.

Keywords: Der 1, soft toys

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Introduction

Evidence exists to suggest that avoidance of allergen exposure can reduce not only asthmatic symptoms but also bronchial hyperreactivity which is a hallmark of asthma [1–5]. Dust mite (*Dermatophagoides*) allergens are known to be a worldwide problem [6,7]. The effort to reduce house dust mites in homes is the basis of asthma treatment. Carpets, mattresses, pillows and bedding in homes of asthmatics must be washed and vacuum cleaned regularly; another option is to change or even completely remove these items from the home. Soft toys also contain nests of dust mites. The close attachment of children to those soft

toys, however, is sometimes so strong that paediatricians and parents have a difficult time removing these toys from them. The special objects, including soft toys, to which children form a strong attachment are termed transitional objects [8]. Paediatric psychotherapists have found cases in which removal of those soft toys revealed later psychological problems [8].

Epidemiological studies from Germany, France, Australia, the UK and the United States have confirmed that levels of mite exposure of $2 \mu\text{g}$ or $10 \mu\text{g}$ of group 1 allergen (Der p 1, Der f 1, Der m 1 and Eur m 1) per gram of dust are relevant to asthma [9–13]. Here we analysed the level of Der 1 (Der p 1 and Der f 1) in old toys, and the level in new toys during seasonal changes. We also compared the effects of washing and vacuum cleaning of old toys on Der 1 levels.

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Materials and methods

Old toys

One hundred and seventy-four old toys which were kept between 1 and 7 years in Japanese households in Tokyo were examined. The toy weight was 220 ± 2.5 g (mean $\pm$ SEM). These toys have been neither cleaned nor washed since they were purchased from the store. About 6% of those toys were kept by families with asthmatic children and/or children with atopic dermatitis.

New toys

Thirty new toys were purchased from a toy store. The toy weight was 24.6 ± 4.1 g (mean $\pm$ SEM) and height 24.6 ± 4.1 cm (mean $\pm$ SEM). They were used for the 1-year study.

Sample collection

Dust from toys was collected with a hand-held vacuum cleaner (power 198 W, airflow rates $0.60 \text{ m}^3/\text{min}$, HC-V10, National, Osaka, Japan) with a filter holder unit in front of the motor unit. Dust was collected using a double layer filter bag (PHC-PA2K, National, Osaka, Japan). Toys were gently vacuumed for 3 min by the same investigator in a consistent manner throughout the study. The paper dust filter bag was weighed before and after 3 min of dust collection. The difference between the two measurements was regarded as the weight of collected dust.

The filter bag was cut into 5 cm (length) $\times$ 1.5 cm (width) strips and then put into 50 mL plastic test tubes. The allergens were extracted with 20 mL of 0.125 M NH_4HCO_3 supplemented with 0.01% bovine serum albumin (BSA) for 2 h at room temperature on a shaker and then kept still overnight at 4°C [11]. The supernatant was collected by squeezing and then lyophilization. Samples were stored at -40°C until assay.

Der 1 assay

Lyophilized samples, reconstituted in 1.5 mL of phosphate buffered saline (PBS) containing 0.05% Tween 20 and 1% BSA were used for Der 1 assay. Briefly, microtitre plate wells coated with a species-specific anti-Der p 1 or anti-Der f 1 monoclonal antibody were incubated with dust extracts and then biotinylated monoclonal antibody directed against a different epitope. The bound allergen-biotinylated monoclonal antibody complex was detected by the addition of streptavidin- β -d-galactosidase conjugate followed by a colour reaction [12]. The summed values for Der p 1 and Der f 1 assayed separately were considered as the total Der 1 levels.

Der 1 changes in new soft toys in the first year

The 30 newly purchased soft toys were divided into three groups. Toys were kept in typical households with children in Setagaya, a residential area of Tokyo. About 7% of those participating families had asthmatic children. The purpose of this study was explained to the parents and they were asked not to change their lifestyle, particularly their house cleaning methods and cleaning intervals, throughout the study. No washing nor cleaning of toys was allowed during the study. The first group started January (winter) 1992, the second group, May (spring) 1992, and the third group, September (end of summer) 1992. Toys were collected every 4 months and dust samples were obtained by the method described above and then returned to each family.

Washing of old toys

Old toys were placed in water containing detergents, polyoxyethylene alkyl ether, alkyl sulfonic acid (sodium ester), and alkyl benzene sulfonic acid (sodium salt) for 1 h and then gently hand-washed for 5 min. After rinsing with water for

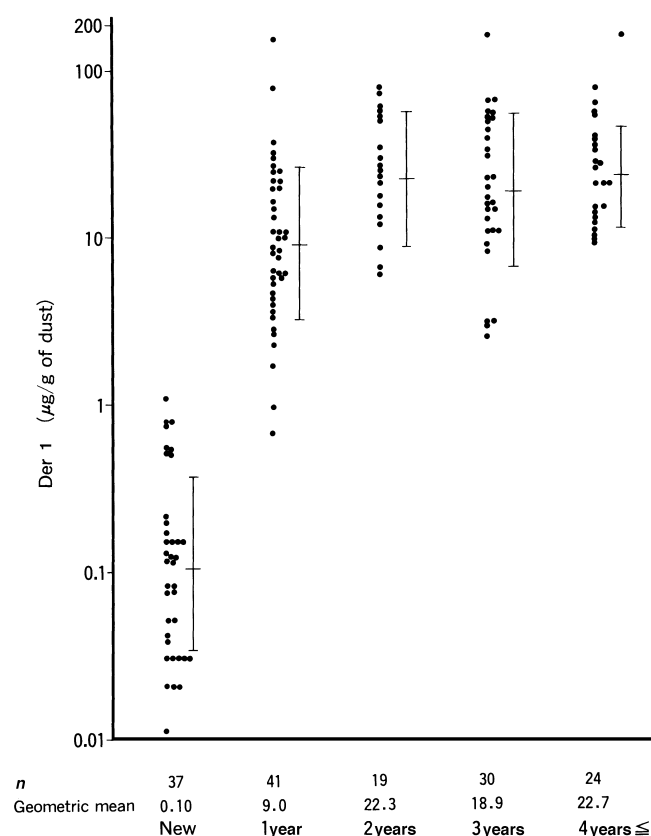


Fig. 1. Der 1 ($\mu\text{g/g}$ of dust) in 37 new soft toys and 172 old soft toys was examined. Each point represents Der 1-value of a toy; mean $\pm$ SD is also shown.

10 min they were wrapped in a bath towel and put into a washing net and then gently dehydrated in an rotating clothes dehydrator at 20°C for 5 min. They were further dried in the laboratory until their weight returned to the baseline. Total time course was 7–10 days.

Statistical analysis

Statistical analysis was performed by Mann–Whitney *U*-test and $P < 0.05$ was regarded as significant.

Results

Weight of dust samples

The mean value of collected samples from toys was 97.5 mg (95% confidence interval, 29.5–169.3 mg). There was no statistical difference between the new and old toys.

Der 1 in new and old toys

Figure 1 shows the mean value of Der 1 (Der f 1 and Der p 1) in new toys was 0.10 $\mu\text{g/g}$ of dust (95% confidence interval, 0.009–1.5 $\mu\text{g/g}$ of dust, $n = 37$), in toys used for 1 year it was 9.00 $\mu\text{g/g}$ of dust (1.03–78.65 $\mu\text{g/g}$ of dust, $n = 41$), in toys used for 2 years it was 22.23 $\mu\text{g/g}$ of dust (3.17–156.84 $\mu\text{g/g}$ of dust, $n = 19$), in toys used for 3 years

it was 18.90 $\mu\text{g/g}$ of dust (2.15–165.84 $\mu\text{g/g}$ of dust, $n = 30$), and in toys used for more than 4 years it was 22.37 $\mu\text{g/g}$ of dust (5.23–98.79 $\mu\text{g/g}$ of dust, $n = 24$).

Der 1 changes in new toys for 1 year

Figure 2 illustrates group 1 who started January (winter) 1992, group 2 who started May (spring) 1992 and group 3 who started September (end of summer) 1992. There was a statistically significant increase in Der 1 in all three groups at 4th, 8th and end of 1 year. However, a variation in Der 1 which did not correlate with the seasons was found.

Comparison of Der 1 between the washed and the vacuum cleaned groups

As shown in Fig. 3 Der 1 in the vacuum cleaned group showed no significant change from 15.3 ± 6.8 $\mu\text{g/g}$ of dust (mean $\pm$ SD) to 11.2 ± 4.0 ; the washed group there was a statistically significant ($P < 0.001$) decrease in Der 1 from 14.3 ± 6.1 – 1.5 ± 0.3 .

Discussion

We measured the major *Dermatophagoides* allergen, Der 1 (Der p 1 and Der f 1) in old toys kept by Japanese families in Tokyo and found that Der 1 increases in houses to form a

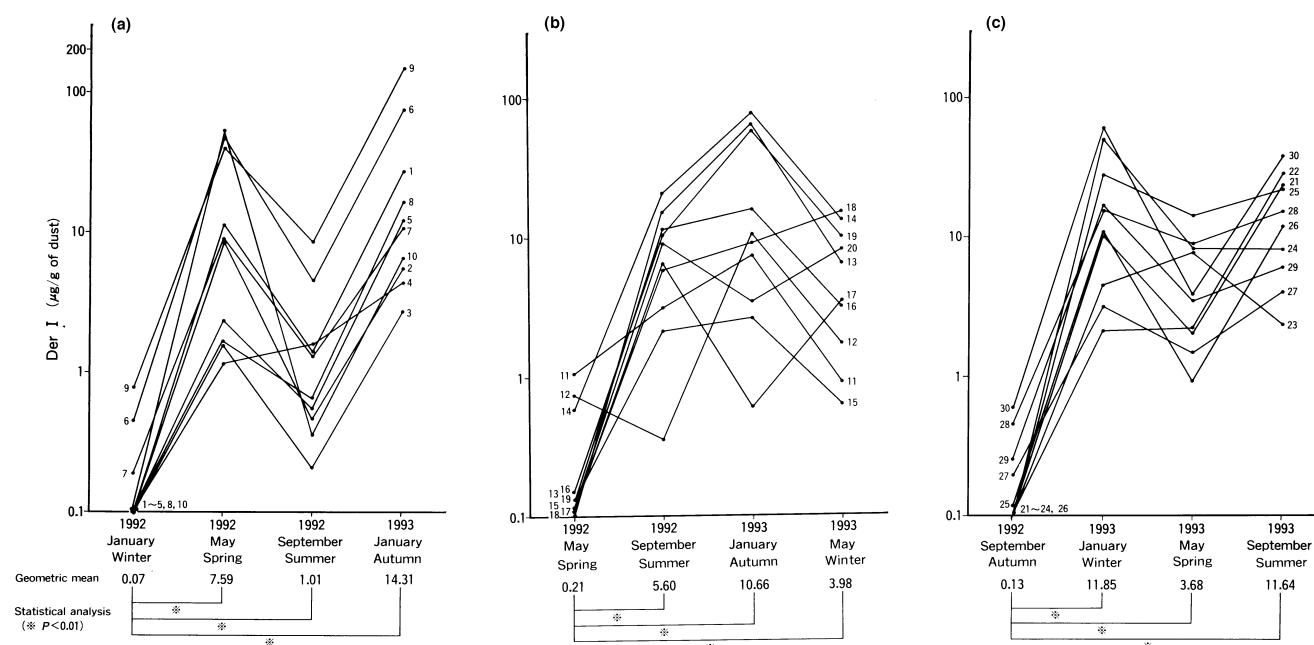


Fig. 2. One-year study of Der 1 in new toys. Toys, No.1–10, were started from January (winter) 1992 (a), No.11–20 from May (spring) 1992 (b), and No.21–30 from September (end of Summer) 1992 (c). Der 1 was measured every 4 months. Statistical analysis was performed by Mann–Whitney *U*-test and $P < 0.05$ was regarded as significant.

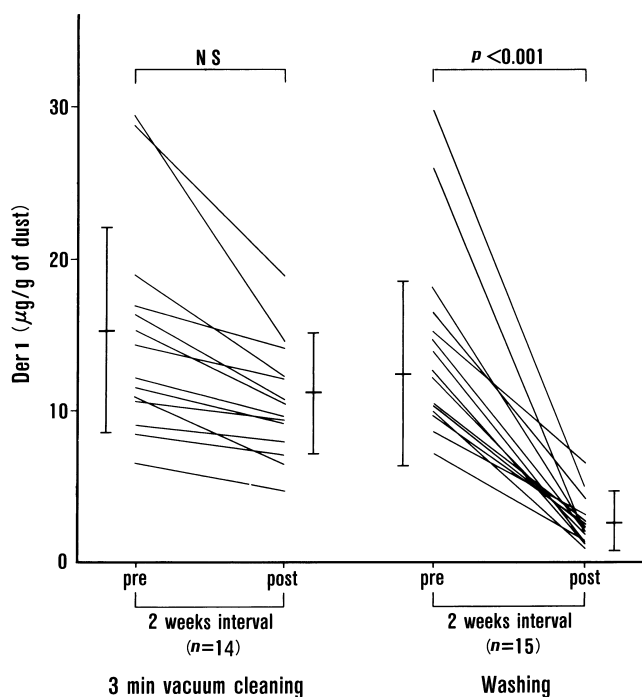


Fig. 3 Comparison of Der 1 in old soft toys between the washed and the vacuum cleaned groups. Left side represents the 3-min vacuum cleaned group ($n = 14$) and the right represents the washing group ($n = 15$). Der 1 was measured after 2 weeks. Statistical analysis was performed by Mann–Whitney *U*-test and $P < 0.05$ was regarded as significant.

potentially important antigen. We also analysed the seasonal variation in Der 1 in new toys kept in volunteer families for 1 year and found that there was no clear seasonal variation in the first year. There was, however, a prominent increase in Der 1 in the first 4 months, indicating that environmental factors in homes had a distinct influence on mites growing in new toys. Humidity, warm houses and cuddling by children are some likely factors involved.

Regarding the effect of cleaning, the washing of soft toys by chemical detergent was compared with 3-min vacuum cleaning. Washing removed a greater amount of mite allergen; there was a statistically significant difference in cleaning effects between the two methods. Washing is thus recommended for cleaning soft toys. Although the alleviation of dust mites in toys obviously does not greatly reduce the total amount of Der 1 allergen in the home, these toys should not be overlooked as they are a significant source of dust mite allergen.

We collected dust samples from soft toys by using an electric hand-held vacuum cleaner for 3 min. The quantity of dust samples collected from soft toys was very small in some cases such as 20 mg. Therefore, we did not use a sieve in order to obtain a sufficient amount of dust for further

analysis. According to the 1990 report of the Second International Workshop, sieving is not obligatory as the fibre mass in dust retains a large fraction of the allergen and this fibre mass is affected by sieving in unpredictable ways [16]. In the present study we collected dust filter bags, carefully cut them into strips and then placed the strips in plastic tubes in order not to lose any of the collected samples before further extraction took place.

For mite avoidance studies, expression of allergen as $\mu\text{g/g}$ of dust fails to indicate the quantity present under certain circumstances. We calculated Der 1/toy in this study and noted some tendencies in Der 1 changes.

The seasonal change in Der 1 in new toys was measured in three different groups over a 1-year period, starting from January (winter), May (spring) and September (end of summer) 1992. There was a prominent increase in Der 1 in all three groups in the first 4 months, indicating that 4 months is sufficient for Der 1 in new toys to reach a dangerous range for asthmatic children. It is hot and moist in Tokyo in the summer which provides favourable conditions for house dust mites to grow. Der 1 in carpets and bed mattresses used for several years tend to increase in the summer and decrease in the winter. We, however, could not find such perennial changes in Der 1 in new toys within the first year. Participating families were selected in a randomized fashion; about 7% of these families (2/30) have asthmatic children which coincides with the general distribution of asthmatic children, suggesting that our results are likely to show the general tendency in Japan. Further we found a wide individual toy variation in Der 1 changes throughout 1 year. We examined the background environmental factors of participating families including carpets, feather mattresses, pets, feather pillows, smoking habit of parents and wooden houses or cement buildings. There was no specific factor in houses to interpret these results. Another possibility is that the seasonal variation in Der 1 might be observed when the level of Der 1 in toys reaches the plateau.

Freezing in a domestic fridge-freezer for 24 h is a convenient and efficient method of killing mites, but does not remove allergens [17]. Therefore we did not try the freezing procedure in this study.

Washing toys is recommended to reduce mite allergen, however, this procedure could possibly damage toys. Soft fur can be damaged and the eyes often come off after repeated washing. In order to keep the soft fur, treatment of these toys with a hair rinse liquid after washing is recommended. Purchasing well-made toys which tolerate repeated washing is also recommended. Toys with an outer covering of nylon are good to avoid mites, however, most children prefer toys which are soft and furry. We are now undertaking a control study to measure Der 1 of toys made of anti-mites textiles (Kanebo Ltd, Tokyo, Japan) in which

microcapsuled anti-mites chemical is inter-woven to determine whether these toys harbour fewer dust mites.

We have found that the new toys become an important source of mite allergen within 4 months after purchase and that a washing is effective to clean the toys. However, we can not make a recommendation as to the ideal washing interval of toys which prevent mite pollution in homes from the present study. This question remains for a further study.

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