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Biologic activity of *Dermatophagoides siboney* and *Blomia tropicalis* allergens in exposed and unexposed mite-allergic individuals. Effect of patient selection on the biologic standardization of mite extracts

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Key words: allergens; biologic standardization; *Blomia tropicalis*; *Dermatophagoides siboney*; domestic mites; skin prick test.

Background: This study aimed to investigate the influence of patient selection criteria, i.e., mite-allergic individuals exposed and not exposed to *Dermatophagoides siboney* and *Blomia tropicalis*, on the biologic activity of mite extracts. Determination of the potency of mite extracts *in vivo* requires selection of patients with a clinical history of mite allergy. In Scandinavia, there are some anamnestic criteria for mite allergy, whereas in the tropics, where patients are continuously exposed to high levels of mites, selection of patients with mite allergy by clinical history is difficult.

Methods: A total of 210 Cuban asthmatics with continuous symptoms, and 43 Swedes with a clinical history of mite allergy were investigated. Skin prick tests were performed with *D. siboney*, *D. pteronyssinus*, *D. farinae*, *B. tropicalis*, *Acarus siro*, *Lepidoglyphus destructor*, and *Tyrophagus putrescentiae* extracts. For analysis of the biologic activity of mite extracts, Cuban patients were divided into four groups:

- 1) all patients skin-test-positive to mites
- 2) patients positive to mites, but not to other inhalant allergens
- 3) patients reacting most to the mite species analyzed
- 4) patients reactive only to mites and reacting most to the mite species analyzed.

The biologic potency was calculated according to the Nordic Guidelines.

Results: Due to cross-reactivity between mites, Swedish mite-sensitive patients, with a clear clinical history of mite allergy, but not exposed to *D. siboney* and *B. tropicalis*, were more skin reactive to these mites than were Cubans. The estimated potency increased gradually to >200% in group 4. In group 1 Cubans, the reactivity to all mites but *B. tropicalis* was lower than that in mite-sensitive Swedes.

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Conclusions: According to the influence of patient selection criteria on the estimation of the potency of mite extracts, the determination of the biologic activity of allergenic extracts in subjects without a clear-cut clinical history should be replaced by new methods when available.

Biologic standardization (BS) by skin prick test (SPT) is recommended in the Nordic Guidelines for registration of allergen preparations (1). The concept implies that the biologic potency of allergenic extracts from different species, labeled with the same biologic units (BU)/ml, should be similar in equally sensitive patients (2). Patient inclusion criteria in BS should be clinical symptoms at exposure and a positive SPT or *in vitro* IgE test to the offending allergen. Reproducible results have been reported from Europe (2). If there is no obvious clinical history, the selection will be mainly based on diagnostic tests, and the sensitivity of these tests will determine the inclusion of patients (3).

In the tropics, where there is a continuously high level of exposure to mite allergens, symptoms of asthma and rhinitis are present throughout the year in atopic patients. Such patients are sensitized to many inhalant allergens. Therefore, selection of mite-sensitive patients, based on the clinical history, is very difficult.

Dermatophagoides pteronyssinus (Dp) and *D. farinae* (Df) are found in temperate as well as in warm and humid climates (4). Other mites such as *D. siboney* (Ds) and *Blomia tropicalis* (Bt) are restricted to tropical countries (5–7).

This study aimed to investigate the influence of patient selection criteria on the biologic activity of Ds and Bt extracts.

Material and methods

Patients

Two populations were investigated:

- 1) 210 Cuban asthmatics (5–52 years of age, mean 28) showing continuous symptoms of asthma and exposed to Ds, Dp, and Bt, but not to Df, which is not found in Cuba
- 2) 43 mite-sensitive Swedish adults (17–67 years of age, mean 33) with rhinitis and/or asthma and clinical history indicating possible mite allergy, i.e., symptoms during the night, symptoms in the morning, and/or seasonal variations with worsening of symptoms in the autumn. They were exposed to Df and Dp, but not to Ds and Bt. Exposure to tropical

mites was excluded by a questionnaire.

All patients gave their informed consent. The ethics committee of the University of Lund approved the part of the study performed in Sweden.

Allergen extracts

Freeze-dried Ds and Bt (7, 8) were used for SPT. Glycerinated extracts of Dp, Df, dog, cat, *Lolium perenne* (Soluprick® SQ, 10 HEP), *Acarus siro* (As), *Lepidoglyphus destructor* (Ld), *Tyrophagus putrescentiae* (Tp) (Soluprick® 1000 BU/ml), *Cladosporium herbarum*, *Aspergillus fumigatus*, and *Penicillium notatum* (Soluprick 1/20 w/v) were obtained from ALK-Abelló, Denmark.

Skin prick tests

SPT was performed in both Swedes and Cubans according to the EAACI guidelines (9). Swedish patients were tested with Ds, Bt, Dp, Df, As, Tp, and Ld extracts, and Cubans with the same extracts except Tp and, in addition, with dog, cat, *Cladosporium*, *Aspergillus*, *Penicillium*, and *Lolium* allergens.

Calculation of biologic activity

For evaluation of the possible influence of patient selection criteria on the biologic activity of extracts, four subgroups of the Cuban patients were analyzed separately:

- 1) all patients who were skin sensitive to mites, i.e., nonselected patients
- 2) those patients who were mite-sensitive without a positive SPT to any other inhalant allergen
- 3) those patients who were most sensitive to the mite species analyzed
- 4) those patients positive only to mites and most reactive to the mite species analyzed.

The biologic potency of each extract in each patient was calculated according to Dreborg (10) by the following formula:

$$\text{BU} = 10\,000 \times \left(\frac{\text{area of allergen wheal}}{\text{area of histamine wheal}} \right)^{2.5}$$

The potency in BU/ml (HEP) of each extract was first calculated in all the patients. Then, the potency of the extracts, i.e., the median BU of the sample, was calculated for each patient group separately.

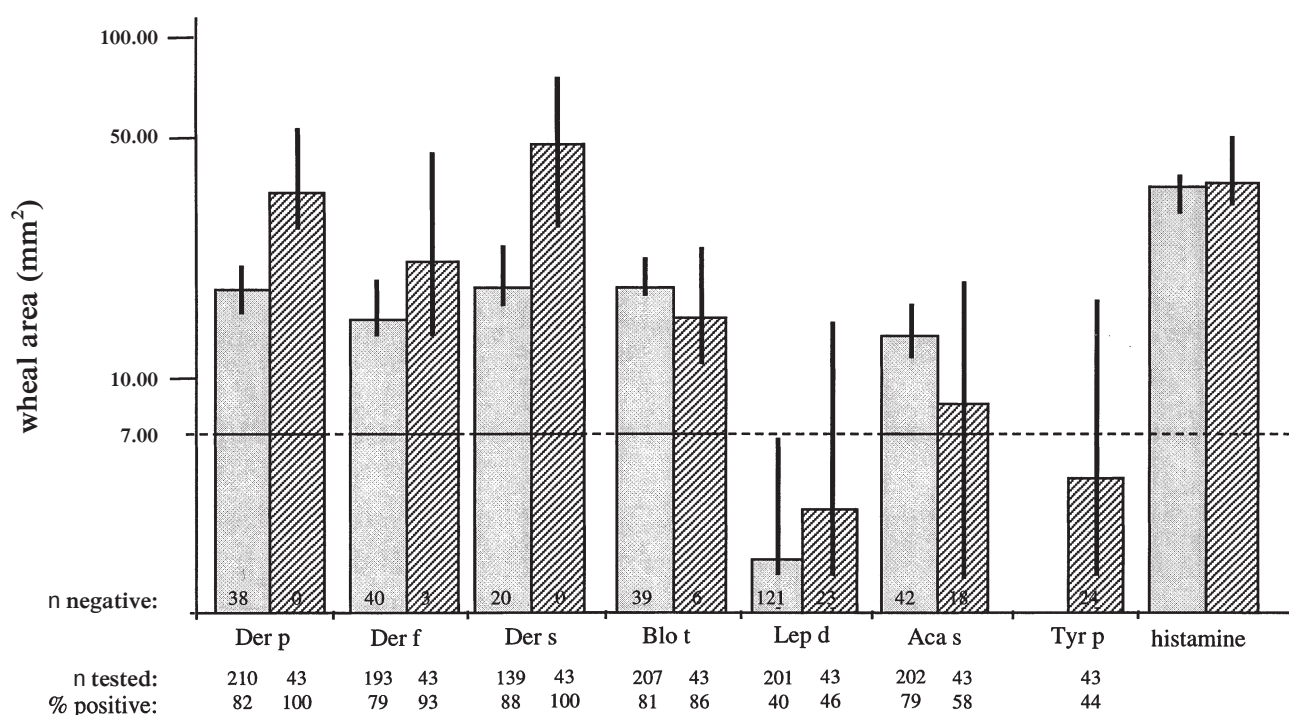


Figure 1. Median size of SPT to different mite extracts in asthmatic Cuban (■) and Swedish (▨) mite-allergic adults. Vertical lines show 95% confidence intervals (CI) of medians.

The nonparametric Mann–Whitney test was used to analyze the differences in the potency between the groups.

Results

The prevalence of sensitization, as measured by positive skin tests to mite allergens, in Cubans and Swedes, is shown in Fig. 1. For all *Dermatophagoides* species, the prevalence degree of positive reactions in Swedes was higher than that in Cubans. In contrast, the skin reactivity to *Bt* was higher in the Cubans than in the Swedes.

The labeled potency of the *Dp* and *Df* extracts, 10 HEP, was confirmed in the Swedish patients selected according to the Nordic Guidelines. The potency in BU of *Ds*, *Df*, *Dp*, and *Bt* extracts, as calculated with results from Swedes and Cubans, is shown in Fig. 2. The *Ds* extract showed the highest potency in the Swedish group. The potency of the *Bt* extract was low, as calculated in the Swedish patients, despite the high prevalence of sensitization.

For all mite allergens, the potency was higher when calculated with data from the subsamples of Cuban patients that best met the selection criteria according to the Nordic Guidelines. In group 1, the “all individuals” group, 82% of the patients also reacted to the other inhalant allergens tested. Group 2 had a biologic activity 66% higher than that

of group 1. All four extracts showed an increased potency of more than 200% in Cubans from groups 1 to 4. The potency of all mite extracts was higher when calculated with Swedes than group 1 Cubans.

The *Df* extract had a lower potency in the nonselected Cubans than the labeled potency of the extract as calculated by the manufacturer. The *Bt* extract had a low potency in nonselected Cubans, and in the Swedish sample of mite-sensitive patients. The *Ds* and *Dp* extracts showed the highest potency in the Swedes, whereas the highest potency of the *Df* and *Bt* extracts was found in the group 4 Cubans.

Discussion

The pattern of sensitization of Cuban asthmatics to mites largely resembled that reported previously (7). All Swedish patients reacted to *Dp* and the majority to *Df*. The nonselected Cubans also reacted to these mite extracts, but to a lesser extent. Furthermore, Swedish patients reacted not only to these extracts but also to *Ds*, and most of them also to *Bt*, i.e., mites not found in Scandinavia.

The Swedish patients met the inclusion criteria of the Nordic Guidelines. In Cuba, we could not define the clinical history of mite sensitivity better than “sensitive to house dust”, meaning indoor allergens, or “perennial allergic

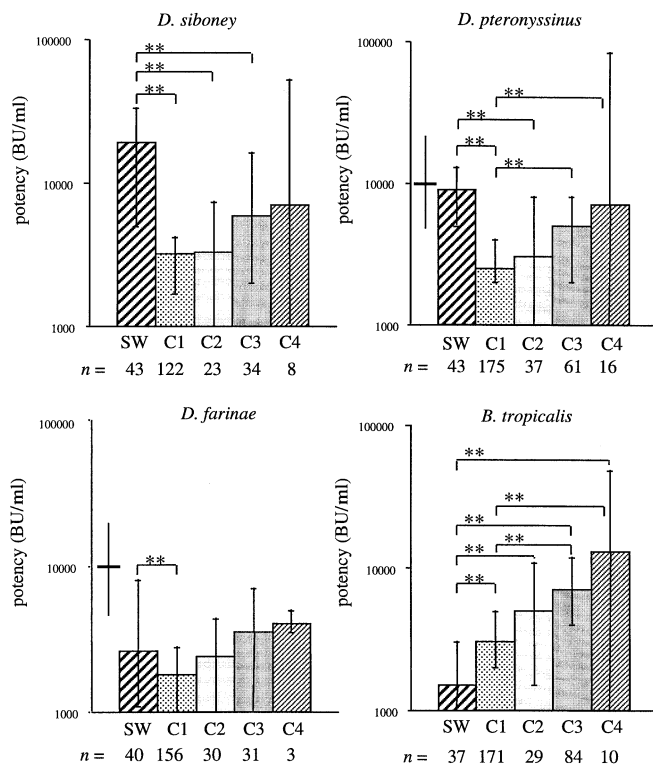


Figure 2. Calculated potency (BU/ml) of mite extracts in Swedes (SW) and in four groups of Cubans (C1–C4). Vertical lines represent 95 % CI of medians. Significant differences are indicated by ** ($P < 0.01$). Solid horizontal lines in *Df* and *Dp* graphs represent labeled potency and tolerance limits of these extracts.

airway symptoms". Moreover, in Cuba, patients are continuously exposed to mites and many other perennial allergens. Thus, symptoms do not vary with the season. Diagnostic tests were used to identify mite-sensitive patients. The sensitivity of the SPT, i.e., the criteria for selection of patients, varies with the potency and composition of the allergenic extract used and with the SPT technique (10). Thus, the potency of the extract varies with the sensitivity of the patients selected for testing.

In Scandinavia, on the other hand, there is some seasonal variation in mite exposure and mite-related problems (11). Thus, mite-allergic patients can be identified by clinical history and selected for biologic standardization by this history in addition to positive SPT or *in vitro* IgE test. With these inclusion criteria, the results of biologic standardization of mite extracts have been shown to be repeatable between regions of Europe (2).

When we used the Swedish patients for biologic standardization, the 95 % CI of the median potency of the *Dp* and *Df* extracts exceeded the tolerated variation in potency in relation to the labeled potency; i.e., 50–200% of the labeled potency, as defined by the Nordic Guidelines (1) and the

Notes for Guidance on Allergen Products of the European Union (12). However, when we calculated the results of all SPT-positive patients (group 1) in the Cuban sample, the activity of these extracts was significantly lower than with the Swedes and lower than the labeled potency. We think the discrepancy occurred because the Cuban patients were not as clinically sensitive to mites as the Swedes. Because we included only the Cuban patients most sensitive to each of the mite species, the median activity approached that obtained in the Swedish sample; i.e., a sample with proper selection criteria.

The extracts of *Ds* and *Bt*, two mites that are not present in Sweden, behaved differently. *Bt* induced weak reactions in the Swedish sample. However, the percentage of positive reactions in Swedes was high. IgE binding to several *Bt* allergens in sera from Swedish allergic subjects has been recently reported, and cross-reactivity with *Ld* has been demonstrated (13). Because of the low reactivity to the *Ld* extract used in our study, the reactivity of the Swedes to *Bt* was possibly explained by cross-reactivity with *Dermatophagoides* species (14, 15). That patients reacted at all to *Ds* must be due to the reported cross-reactivity between *Ds* and the mite species found in Scandinavia (15). The *Dp* and *Df* extracts had a potency adjusted (diluted) to 10 000 BU/ml, whereas mite extracts prepared without adjustment of the potency but prepared according to modern principles, such as the *Bt* and *Ds* extracts, are mostly much more potent (16).

In conclusion, biologic standardization cannot be achieved if the patient inclusion criteria cannot be strictly followed. If this is not possible, other methods of potency adjustment must be sought. Determination of major allergen content, which is commonly done, has been shown to reflect the biologic activity of allergenic extracts (17) and has been recommended for biologic standardization (18). However, this method does not always give correct information about the biologic activity in man, since the monoclonal antibodies used in such assays probably do not often recognize all isoallergens and thus do not determine the total amount of allergen present. Furthermore, they often do not recognize IgE epitopes; therefore, they do not determine the biologic activity of extracts.

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References

1. Nordic Council on Medicines. *Registration of allergen preparations. Nordic Guidelines*. NLN Publication no. 23. 2nd ed. Uppsala, Sweden, 1989.
2. Dreborg S, Basomba A, Belin L, et al. Biological equilibration of allergen preparations: methodological aspects and reproducibility. *Clin Allergy* 1987;**17**:537–550.
3. Dreborg S, Sjögren I, Eriksson NE, Einarsson R. Selection of patients for biological standardization as exemplified by standardization of mugwort, goosefoot and English plantain pollen allergen extracts/preparations. *Allergy* 1987;**42**:485–495.
4. Baena-Cagnani CE, Patino CM. Mite allergen exposure in Latin America. In: Johansson SGO, editor. *Progress in allergy and clinical immunology*. Vol. 3. Stockholm: Hogrefe & Huber, 1995:330–333.
5. Fernández-Caldas E, Lockey RF. *Blomia tropicalis* – a mite of clinical importance. *Allergy Clin Immunol News* 1995;**7**:39–42.
6. Fernández-Caldas E, Sepulveda A, Montealegre F. Identification of the domestic mite fauna in Puerto Rico. *Allergy* 1995;**50 Suppl 26**:72.
7. Ferrándiz R, Casas R, Dreborg S. Sensitization to *Dermatophagoides siboney*, *Blomia tropicalis*, and other domestic mites in asthmatic patients. *Allergy* 1996;**51**:501–505.
8. Ferrándiz R, Casas R, Dreborg S, Einarsson R, Bonachea I, Chapman M. Characterization of allergenic components from house dust mite *Dermatophagoides siboney*. Purification of Der s 1 and Der s 2 allergens. *Clin Exp Allergy* 1995;**25**:922–928.
9. European Academy of Allergology and Clinical Immunology. Skin tests used in type I allergy testing. Position paper. *Allergy*; 1989;**44 (Suppl 10)**.
10. Dreborg S. The skin prick test. Methodological studies and clinical applications. Linköping University Medical Dissertation no. 239, 1987, 239.
11. Munir AK, Einarsson R, Kjellman NI, Björkstén B. Mite (Der p 1, Der f 1) and cat (Fel d 1) allergens in the homes of babies with a family history of allergy. *Allergy* 1993;**48**:158–163.
12. European Agency for the Evaluation of Medicinal Products, Committee for Proprietary Medicinal Products (CPMP). Note for guidance on allergen products, 1996.
13. Johansson E, Schmidt M, Johansson SGO, Machado L, Olsson S, van Hage-Hamsten M. Allergic cross-reactivity between *Lepidoglyphus destructor* and *Blomia tropicalis*. *Clin Exp Allergy* 1997;**27**:691–699.
14. van Hage-Hamsten M, Machado L, Barros MT, Johansson SGO. Immune response to *Blomia kulagini* and *Dermatophagoides pteronyssinus* in Sweden and Brazil. *Int Arch Allergy Immunol* 1990;**91**:186–191.
15. Ferrándiz R, Casas R, Dreborg S, Einarsson R, Fernández B. Crossreactivity between *Dermatophagoides siboney* and other house dust mite allergens in sensitized asthmatic patients. *Clin Exp Allergy* 1995;**25**:929–934.
16. Dreborg S, Einarsson R, Longbottom J. The chemistry and standardization of allergens. In: Weir DM, editor. *Handbook of experimental immunology*. London: Blackwell Scientific, 1986:1–10, 28.
17. Dreborg S, Einarsson R. The major allergen content of allergenic preparations reflects their biological activity. *Allergy* 1992;**47(4 pt 2)**:418–423.
18. American Academy of Allergy, Asthma, and Immunology. The use of standardised allergen extracts. *J Allergy Clin Immunol* 1997;**99**:583–586.