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# Sensitization to *Dermatophagoides pteronyssinus* and *Blomia tropicalis* Extracts and Recombinant Mite Allergens in Atopic Thai Patients

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**SUMMARY** **Mite surveys in Thailand indicated that *Dermatophagoides pteronyssinus* (Dp) is predominant, but so far there were no data available on *Blomia tropicalis* (Bt), which is prevalent in the Asia Pacific region. Skin prick testing (SPT) was performed in 40 atopic children, 45 atopic adults and 17 non-atopic volunteers. Skin reactions to Dp were found in 25/40 (62.5%) and 23/45 (51.1%); skin reactions to Bt were found in 15/40 (37.5%) and 18/45 (40%) in atopic children and adults, respectively. SPT to the major sensitizing allergens Der p 1, Der p 2, Der p 5, and Blo t 5 showed positive results in 14/40 (35%), 12/40 (30%), 1/40 (2.5%) and 4/40 (10%) of atopic children, and in 12/45 (26.7%), 13/45 (28.9%), 5/45 (11.1%), 6/45 (13.3%) of atopic adults, respectively. The results indicate that Dp is one of the major sources of allergy, while Bt is a minor one and that Der p 1 and Der p 2 are important mite allergens in Chiang Mai, Thailand.**

Studies from different parts of the world have confirmed the association between house dust mite allergy and the increasing prevalence of asthma and allergic diseases.[1] The pyroglyphid mites *Dermatophagoides* are the main sources of house dust allergens.[1] The non-pyroglyphid storage mites have been shown to cause symptoms like asthma, allergic rhinitis and conjunctivitis in a rural occupational environment, and recently, in urban populations as well.[2] Among the storage mites, *Lepidoglyphus destructor* is the predominant species in northern and central Europe, whilst the *Blomia* genus is the most prevalent in tropical and sub-tropical parts of the world.[3-5] Studies show that *Dermatophagoides pteronyssinus* (Dp) and *Blomia tropicalis* (Bt) are endemic in Asia Pacific countries such as Singapore and Taiwan.[5,6] In Thailand, the most abundant species is Dp.[7] Through the progress in molecular biology of allergens, all the important major and minor allergens have been cloned and produced as recombinant allergens (rAllergens).[8] Studies on the sensitization to Dp and Bt rAllergens suggested that Der p 1, Der p 2 and Blo t 5 are the major sensitizing allergens in Singapore and Taiwan.[6,9,10] Skin prick testing (SPT) in children with asthma in Bangkok and Chiang Mai confirmed that most of the patients have been sensitized to Dp.[11-13] Little is known about the role of Bt and other important house dust mite allergens in allergic diseases in Thailand. In this study, the skin reactions to Dp and Bt extracts, and also the skin reaction profiles to Dp and Bt rAllergens in atopic patients and non-atopic controls in Chiang Mai were evaluated. The protocol of this study was approved by the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University.

## MATERIALS AND METHODS

### Subjects

Forty children (aged 5-14 years old), 45 adults (aged 15-52 years old) with atopic diseases (asthma and/or allergic rhinitis and/or atopic dermatitis), and 17 non-atopic adults (aged 16-48 years old) were recruited into this study. All subjects or guardians signed the informed consent before entering the study. Each participant was examined and interviewed to assess the presence of atopic diseases by the physician

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investigator. Persons with atopic dermatitis were defined as those with chronic or relapsing pruritic rash in the flexural areas of the limbs or around the neck (adapted from Hanifin and Rajka diagnostic criteria for atopic dermatitis). Asthma was determined by a physician's diagnosis report of asthma or complaints of frequent wheezing problems in the past twelve months. Allergic rhinitis was defined as frequent paroxysms of sneezing, nasal pruritus, nasal congestion or profuse watery nasal discharge that did not result from upper respiratory tract infections during the past twelve months.

Non-atopic controls were adult volunteers who were healthy and did not have history or symptoms and signs of atopic diseases as stated above.

Individuals were evaluated when they did not have symptoms and were not taking antihistamines.

### Allergens

Allergens used in this study included the following:

1. Natural mite extracts:

   1.1 *Dermatophagoides pteronyssinus*, 10,000 AU/ml (Greer Laboratories, NC, USA)

   1.2 *Blomia tropicalis*, 0.2 mg/ml (Allergy Laboratory of the National University of Singapore, Singapore)[9,14]

2. Native Der p 1 (25 μg/ml), and recombinant mite allergens, 25 μg/ml, including Der p 2, Der p 5, Blo t 4 expressed in *Pichia pastoris*, and Blo t A2, Blo t 3, Blo t 5, Blo t 6, Blo t 10, Blo t 11, Blo t 12, Blo t 13 expressed in *Escherichia coli* (Allergy Laboratory of the National University of Singapore, Singapore).[9]

3. Controls

   3.1 Positive control: histamine, 1 mg/ml (Center Laboratories, New York, USA)

   3.2 Negative controls:

      3.2.1 50% glycerin/50% buffered saline solution (Greer Laboratories, NC, USA)

      3.2.2 Glutathione-s-transferase (GST) control for the GST fusion protein in rAllergens (Allergy Laboratory of the National University of Singapore, Singapore)[9]

### Skin prick testing (SPT)

SPT was performed using lancets (Feather Safety Razor, Japan) on the volar surface of the forearm as has been described and the reactions were read after ten minutes for histamine, and fifteen minutes for allergens.[15] The results were considered positive when the sum of the widest length and breadth of a wheal with erythema divided by two was over three mm.[15]

### Statistical analysis

The significance of the positive reactions in the atopic group compared with those in the control groups was determined by statistical analysis using the chi-square test with correction.

## RESULTS

The demographic data of all subjects are shown in Table 1. Twenty-five out of the 40 (62.5%) atopic children and 23/45 (51.1%) of the atopic adults reacted to Dp extract (Table 2). Fewer subjects reacted to Bt extract, 15/40 (37.5%) and 18/45 (40%) of atopic children and adults, respectively (Table 3). Four out of the 17 (24%) and 2/17 (11.8%) in the control group reacted to the extracts of Dp and Bt, respectively.

Der p 1 and Der p 2 gave similar numbers of positive reactions in both groups, 14 (35%) *vs* 12 (30%) in atopic children, and 12 (26.7%) *vs* 13 (28.9%) in atopic adults, while a very small number of both groups reacted to Der p 5 (Table 2). Nine out of the 40 (22.5%) atopic children and 11/45 (24.4%) of atopic adults reacted to more than one Dp rAllergen (data

**Table 1** Demographic data of all subjects

| | n | Mean age* (range) | Asthma (%) | AR** (%) | AD*** (%) | Asthma & AR (%) | Male (%) |
|---|---|---|---|---|---|---|---|
| Controls | 17 | 29 (16-48) | 0 | 0 | 0 | 0 | 35 |
| Atopic children | 40 | 10.5 (5 -14) | 90 | 92.5 | 12.5 | 82.5 | 65 |
| Atopic adults | 45 | 24.5 (15-52) | 47 | 96 | 4 | 44.4 | 51 |

*age in years. **allergic rhinitis. ***atopic dermatitis

**Table 2** Positive reactions to skin prick testing against Dp and its rAllergens

| | Dp (%) | Der p 1 (%) | Der p 2 (%) | Der p 5 (%) | Combined Der p 1, 2, 5* |
|---|---|---|---|---|---|
| **Controls** n = 17 | 4 (24) | 0 | 0 | 0 | 0 |
| **Atopic children** n = 40 | 25[a] (62.5) | 14[a] (35) | 12[b] (30) | 1(2.5) | 17 |
| **Atopic adults** n = 45 | 23 (51.1) | 12[b](26.7) | 13[b](28.9) | 5 (11.1) | 14 |

*Combined Der p 1, 2, 5 = number of patients with positive SPT to at least one of Der p 1, Der p 2 and Der p 5
[a] $p = 0.01$ (compared with controls)
[b] $p < 0.05$ (compared with controls)

not shown). Seventeen children and 14 adults reacted to at least one Dp rAllergen (combined positive reactions to Der p 1, Der p 2 and Der p 5) (Table 2).

Reactions to Bt rAllergens, predominantly positive to Blo t 5, were 4/40 (10%) of atopic children and 6/45 (13.3%) of atopic adults. Reactions to the rest of Bt rAllergens were negative, except for one (2.2%) of the atopic adults who reacted to Blo t 4 (Table 3).

**Table 3** Positive reactions to skin prick testing against Bt and its rAllergens

| | Bt (%) | Blo t 4 (%) | Blo t 5 (%) | Combined Blo t 4, 5* |
|---|---|---|---|---|
| **Controls** n = 17 | 2 (11.8) | 0 | 0 | 0 |
| **Atopic children** n = 40 | 15 (37.5) | 0 | 4 (10) | 4 |
| **Atopic adults** n = 45 | 18 (40) | 1 (2.2) | 6 (13.3) | 6 |

Combined Blo t 4, 5 = number of patients with positive SPT to at least one of Blo t 4 and Blo t 5

None of the control group reacted to either Dp or Bt rAllergens (Tables 2 and 3).

The mean wheal diameter of the skin reactions to Dp, Bt and their rAllergens in three groups of subjects are shown in Table 4.

There was no side effect of SPT, either from natural extracts or rAllergens.

## DISCUSSION

Our study confirms the findings from former studies[11-13] that one of the most important allergens associated with atopic diseases in Thai children is Dp ($p = 0.01$, compared with controls). In some tropical countries such as Singapore, sensitization to Bt is predominant,[8] while in other countries, such as Taiwan,[9] Indonesia,[16] and Malaysia,[17] sensitization to Dp is more prevalent. The results of our study showed that sensitization to Bt was not uncommon in Thailand. However, sensitization to Dp was far more common, especially in children. This is in accordance with the result of a mite survey in Thailand, which revealed that Dp was the most abundant species, and dust from several houses surveyed in that study had a significantly high level of group I antigens.[7] Until now, there has been no report on group II

**Table 4** Mean wheal diameter (mm) of the positive skin reactions against Dp, Bt and their rAllergens

| | Dp | Der p 1 | Der p 2 | Der p 5 | Bt | Blo t 4 | Blo t 5 |
|---|---|---|---|---|---|---|---|
| **Controls** | 4.9 (n = 4) | 0 | 0 | 0 | 4 (n = 2) | 0 | 0 |
| **Atopic children** | 7.5 (n = 25) | 5.8 (n = 14) | 5.7 (n = 12) | 5.5 (n = 1) | 5.5 (n = 15) | 0 | 5.9 (n = 4) |
| **Atopic adults** | 7.1 (n = 23) | 6.5 (n = 12) | 8.2 (n = 13) | 4.6 (n = 5) | 5.3 (n = 18) | 4 (n = 1) | 9.6 (n = 6) |

antigens of Dp in Thailand. The association of sensitization to Dp and Bt and atopic diseases in the adult group was not obvious. This might reflect the importance of other inhalant allergens in adults.

One of the problems associated with using natural allergenic extracts for the diagnosis and treatment of allergic diseases is their non-allergenic protein contents. Recently, genetically-engineered rAllergens were produced for many major allergens. These products have defined concentrations and are free of irrelevant proteins.[8] The biologic activities of the mite rAllergens have been established by SPT in allergic patients and non-allergic control subjects.[18,19] Recent studies indicated the presence of both cross-reactive and unique allergens in Dp and Bt,[6] and further evaluation using rAllergens indicated that the cross-reactive allergens in Bt were not closely related to the major Dp allergens.[20] Using rAllergens provides greater specificities and fewer problems with cross-reactivitiy than the use of natural allergen extracts. In the tropics, where helminthic infections are endemic, parasites can nonspecifically potentiate the synthesis of an IgE antibody against common environmental allergens without having evident clinical consequences.[21] Testing with purified allergens discriminates better between a nonsymptomatic atopic state and a clinically relevant allergy.[22] In our atopic adults, whose correlation between atopic diseases and the positive reactions to Dp and Bt extracts were not obvious, the positive reactions to Der p 1 and Der p 2 correlated with the presence of atopic diseases ($p < 0.05$, compared with controls). Our study shows that Der p 1 and Der p 2 are the important allergens for Dp in Chiang Mai, and that the important allergen of Bt is the same as the one in Singapore and Taiwan, which is Blo t 5.

In our atopic patients, the percentage of positive reactions against Bt extract was quite high, but that against Blo t 5 was low. This might be due to some degree of cross-reactivity between Dp and Bt. The low percentage of positive reactions to Blo t 5 (10% in children, 13.3% in adults) indicated that Bt was a minor allergen in Chiang Mai. The results of a recent study in Bangkok on SPT against Bt and its rAllergens were slightly different from ours.[23] Using the same protocol as ours, they showed that the positive skin reactions to crude Bt extract and Blo t 5 were 41% and 21.9%, respectively, in atopic children and adults.

Among 25 atopic children and 23 atopic adults who gave positive reactions against Dp extract, the number of positive reactions to a single Dp rAllergen, Der p 1, or Der p 2, or Der p 5 was 14 (56%) or less in children, and 13 (52%) or less in adults. The diagnostic sensitivity of a single rAllergen is generally lower than that obtained with allergen extracts, but it can be increased considerably by using rAllergen panels covering the most important allergenic structures present in a given complex allergenic extract.[24] In our study, of the subjects who reacted to Dp extract, the combined number of patients with positive reactions against Der p 1, Der p 2 and Der p 5 were 17/25 (68%) in children, and 14/23 (60.9%) in adults. In a recent study,[18] Dp allergic children with asthma and/or allergic rhinitis were tested with rAllergens at 5 μg/ml concentration and patients with a negative test result were further tested with 50 μg/ml. Positive SPT results to Der p 2 and Der p 5 were 82% and 51%, respectively. In a review of 11 studies on mites and 25 studies on other major rAllergens,[24] the authors concluded that the use of rAllergens for skin testing gave an excellent specificity, which often reached 100%, but sensitivity was generally lower than that obtained with commercial extracts. From the available data, they recommended the use of a 100 μg/ml concentration to achieve good sensitivity and keep an excellent specificity in SPT. They also concluded that skin testing with rAllergens did not bear a higher risk compared to that with conventional, commercially available extracts, and false positive skin testing with rAllergens was extremely rare. In our study, the low percentage of positive reactions to rAllergens might be due partly to the low concentration of rAllergens used (25 μg/ml). The negative reaction to rAllergens, despite the positive reactions to Dp and Bt extracts, in some members of our control group also confirmed the high specificity of rAllergens.

In conclusion, we showed that in Chiang Mai, Thailand, sensitization to Dp was associated with atopic diseases in children, and Der p 1 and Der p 2 were the important mite allergens. Sensitization to Der p 1 and Der p 2 was associated with atopic diseases in both children and adults. The low number of positive reactions to Bt rAllergens indicated that Bt was a minor mite allergen. Of Bt allergens, the most important one was Blo t 5. The high number of positive reactions to crude Bt extract might be due to the cross-reactivity between Dp and Bt. This problem may be overcome by using rAllergens that have been shown to give high specificity. Because Dp has been shown to be the most prevalent mite allergen in Thailand, and that Der p 1 and Der p 2 were the important ones, they should be included in the allergen panels for SPT. The results of our study showed that Bt is not uncommon. However, further studies should be carried out to confirm the prevalence of Bt in Thailand and the clinical relevance of these allergens before considering to include Bt

extract or its rAllergens in the allergen panels for SPT.

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