RESEARCH ARTICLE

Co-sensitization and cross-reactivity of *Blomia tropicalis* with two *Dermatophagoides* species in Guangzhou, China

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Abstract

**Background:** Contradictory results have been reported previously in the analyses of cross-reactivity among *Blomia tropicalis* (Blo t), *Dermatophagoides pteronyssinus* (Der p), and *Dermatophagoides farinae* (Der f). This study aims to investigate the characteristics of co-sensitization and the IgE cross-reactivity among them and attempts to identify whether patients are sensitized to Blo t due to cross-reaction or true sensitization.

**Methods:** Specific IgE (sIgE) in the sera from 1497 allergic patients was determined by ImmunoCAP. Cross-reactivity was analyzed and determined by sIgE inhibition with 21 sera samples.

**Results:** Around 85.50% of patients were sensitized to Der p, 85.37% of patients were sensitized to Der f, and 71.54% of patients were sensitized to Blo t. Further, 70.14% of patients were co-sensitized to Blo t, Der p, and Der f, and only seven patients were sensitized solely to Blo t. With increasing sIgE levels for Blo t, the positive rates of severe-level (class 5-6) co-sensitization to Der p or Der f significantly increased. Blo t was moderately associated with Der p and Der f, with correlation coefficients of 0.6998 and 0.6782, respectively. Der p and Der f inhibited IgE binding to Blo t more strongly than Blo t inhibited IgE binding to Der p or Der f in the patient groups $C\_{}^{\text{Blo t}} < C\_{}^{\text{Der p}}$ and $C\_{}^{\text{Blo t}} < C\_{}^{\text{Der f}}$

**Conclusions:** This study has established valuable information about the co-sensitization and cross-reactivity of Blo t with two *Dermatophagoides* species (Der p and...
INTRODUCTION

Mites are a prevalent and important source of allergenic proteins that are associated with allergic respiratory diseases, such as asthma and rhinitis. *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f) are the predominant mite species worldwide. Yet, *Blomia tropicalis* (Blo t) is an important mite allergen in tropical regions like Singapore, Malaysia, Columbia, and the Taiwan and Hainan provinces of China.1–5 Blo t coexists with Der p and Der f, and Blo t-sensitized patients are usually co-sensitized to Der p and Der f, with reports of more than 30% of patients sensitized to all three species.6 Some studies have reported a moderate correlation between Der p and Blo t through analysis of the levels of specific IgE (sIgE).6–8 However, partial IgE inhibition assays have shown that Blo t allergens have little cross-reactivity with Der p and Der f.9–11 At present, studies about cross-reactivity between Blo t and Der p and Blo t and Der f are few and appear to be inconsistent. Whether patients co-sensitized to Blo t and the other two mites are due to true sensitization or cross-reaction is still unknown.

Guangzhou, a capital city of Guangdong province, located in Southern China in sub-tropical monsoon climate region that is suitable for proliferation of dust mites, storage mites, and fungi due to its warm and humid conditions. Though lower levels of Blo t antigens were found in bedding dust samples and living room samples from Guangzhou city,12 it was found that there was a high positive rate (88.2%) of Blo t in asthma patients with or without rhinitis.13 In our study, we investigate the co-sensitization and correlation of Blo t, Der p, and Der f using sIgE measurements. SlgE inhibition was used to evaluate the IgE cross-reactivity among these mites, attempting to identify whether sensitization to Blo t was due to cross-reaction or true sensitization.

MATERIALS AND METHODS

2.1 Ethics statement

This study was approved by the Medical Ethics Committee of The First Affiliated Hospital of Guangzhou Medical University (ethics approval no. gyfyy-2016-73). All experiments were performed in accordance with relevant guidelines and regulations of the Ethics Committee of The First Affiliated Hospital of Guangzhou Medical University.

2.2 Study subjects

Figure 1 shows a flowchart of our study protocol. The Allergy Information Repository of the State Key Laboratory of Respiratory

![FIGURE 1 Study design and flowchart](image-url)
Disease (AIR-SKLRD) is a large serum biobank containing serum from allergic patients along with detailed clinical history and examination records.\textsuperscript{14,15}

There were 1497 sera samples from mite-allergic patients stored in AIR-SKLRD, which were selected to detect sIgE level to Blo \textit{t}, Der \textit{p}, and Der \textit{f} and to analyze the co-sensitization and correlation between them. These patients were sensitized to at least one mite detected by ImmunoCAP method (mite allergen sIgE >0.35 kU/L). The average age was 20.01 ± 17.51 years old (ranging from 1 to 86 years old), and there were 929 males and 568 females.

The 1497 sera samples were divided into three groups according to the sIgE level to Blo \textit{t}, Der \textit{p}, and Der \textit{f}, including group C\textsubscript{Blo \textit{t}} > C\textsubscript{Der \textit{p}} (the class of sIgE to Blo \textit{t} was higher than that of Der \textit{p}, n = 35), group C\textsubscript{Blo \textit{t}} = C\textsubscript{Der \textit{p}} (the class of sIgE to Blo \textit{t} was equal to that of Der \textit{p}, n = 329), and group C\textsubscript{Blo \textit{t}} < C\textsubscript{Der \textit{p}} (the class of sIgE to Blo \textit{t} was lower than that of Der \textit{p}, n = 1133). Of the three groups, a total of 21 sera samples (six from group C\textsubscript{Blo \textit{t}} > C\textsubscript{Der \textit{p}}, six from group C\textsubscript{Blo \textit{t}} = C\textsubscript{Der \textit{p}}, and nine from group C\textsubscript{Blo \textit{t}} < C\textsubscript{Der \textit{p}}) were randomly selected for sIgE inhibition assay. The characteristics of the study subjects selected in the study are shown in Table 1.

### Measurement of sIgE to Blo \textit{t}, Der \textit{p}, and Der \textit{f}

Sera sIgE to Der \textit{p}, Der \textit{f}, and Blo \textit{t} was measured with the ImmunoCAP system (Phadia 1000; Thermo Fisher Scientific Inc.) according to the manufacturer’s instructions. sIgE levels were expressed in kilo units per liter (kU/L), and the detected range of sIgE was 0.1-100 kU/L. Any measurement over the upper limit of the detected range was given a value of 100 kU/L. Tests with sIgE levels lower than 0.35 kU/L were defined as sIgE-negative, otherwise, they were defined as sIgE-positive. sIgE-positive tests were categorized into 6 classes: class 1 (≥0.35-<0.70 kU/L), class 2 (≥0.70-<3.50 kU/L), class 3 (≥3.50-<17.50 kU/L), class 4 (≥17.50-<50.00 kU/L), class 5 (≥50.00-<100.00 kU/L), and class 6 (≥100.00 kU/L). Class 1 and class 2 were considered to be mild sensitization, class 3 and class 4 moderate sensitization, and class 5 and class 6 severe sensitization.

### Crude extract preparation of Blo \textit{t}, Der \textit{p}, and Der \textit{f}

Der \textit{p} (lot number: 327874), Der \textit{f} (lot number: 326781), and Blo \textit{t} (lot number: 287875) allergens were purchased from Greer Laboratories.

### Table 1

| Patients | Gender (M/F) | Age (M, IQR) | sIgE to Der \textit{p} (KU/L) | sIgE to Der \textit{f} (KU/L) | sIgE to Blo \textit{t} (KU/L) |
|----------|--------------|--------------|-----------------------------|-----------------------------|-----------------------------|
| IgE analysis | 1497 | 929/568 | 11 (22) | 40.9 (95.95) | 44.20 (95.87) | 1.57 (5.75) |
| sIgE inhibition | 21 | 16/5 | 11 (12.5) | 57.5 (63.9) | 77.8 (62.55) | 34.3 (52.22) |

| Patients for inhibition assay | M | Age | sIgE to Der \textit{p} (KU/L) | sIgE to Der \textit{f} (KU/L) | sIgE to Blo \textit{t} (KU/L) |
|------------------------------|---|-----|-----------------------------|-----------------------------|-----------------------------|
| P1 | F | 51 | 47.5 | 87.8 | 92.4 |
| P2 | M | 8 | >100 | >100 | 13.1 |
| P3 | M | 59 | 54.3 | >100 | >100 |
| P4 | M | 17 | 73.8 | 69.9 | 10.1 |
| P5 | M | 13 | >100 | 238 | 10.9 |
| P6 | F | 6 | 95.5 | 57.8 | 14.8 |
| P7 | M | 10 | 51.7 | >100 | 11.5 |
| P8 | M | 10 | 72.2 | >100 | 12.3 |
| P9 | M | 9 | 91.8 | 83 | 27.8 |
| P10 | M | 11 | 70.8 | 77.8 | >100 |
| P11 | M | 11 | 2.63 | 2.28 | 58.2 |
| P12 | M | 13 | 9 | 18.12 | 67.04 |
| P13 | M | 7 | 28.5 | 35.6 | 34.3 |
| P14 | F | 61 | 31 | 39.3 | 25.4 |
| P15 | M | 40 | 44.6 | >100 | 82.4 |
| P16 | M | 11 | >100 | >100 | 59.3 |
| P17 | M | 11 | >100 | >100 | 56.1 |
| P18 | F | 12 | 61 | 56.1 | 62.8 |
| P19 | M | 5 | 57.5 | 50.9 | 41 |
| P20 | M | 8 | 17.1 | 17.9 | 10.7 |
| P21 | F | 25 | 13.4 | 11.6 | 14.1 |

Abbreviations: Blo \textit{t}, Blomia tropicalis; Der \textit{f}, Dermatophagoides farinae; Der \textit{p}, Dermatophagoides pteronyssinus; M (IQR), median (interquartile range); M/F, male/female; sIgE, specific IgE.
The allergen lyophilized cakes were dissolved in PBS, aliquoted to Eppendorf tubes, and stored at −80°C until used. Allergen protein concentrations were determined by BCA assay (Pierce™ BCA protein assay).

### 2.5 Allergen sIgE inhibition assay

Each serum sample was diluted to test mite sIgE concentration of approximately 10 kU/L, followed by mixing with equal volume of PBS, Blo t, Der p, and Der f allergen crude extract (2 mg/mL), respectively. After incubating at 37°C and shaking for 1 hour, the sIgE levels were measured by ImmunoCAP, and the sIgE inhibition rate was calculated using the following formula: inhibition rate = (sIgE<sub>PBS</sub> − sIgE<sub>allergen</sub>) / sIgE<sub>PBS</sub> × 100%.

### 2.6 Statistical analysis

Statistical software package SPSS version 19.0 was used to analyze all data. Parametric quantitative data were expressed as the mean ± standard deviation. Non-parametric quantitative data were reported as a median value (interquartile range). Wilcoxon matched-pairs signed-rank test was used to compare the variance of data within groups, while comparison among three groups was performed with the Kruskal-Wallis test. Mann-Whitney U test was used to compare two groups. Correlation analyses between non-parametric data were performed using Spearman’s test, with the correlation coefficients presented as “r.” Differences were considered statistically significant when P values were <.05.

### 3 RESULTS

#### 3.1 Pattern of sensitization among ImmunoCAP positive for Blo t, Der p, and Der f

As the Venn diagram in Figure 2 shows, 85.50% of patients were sensitized to Der p, 85.37% of patients were sensitized to Der f, and 71.54% of patients were sensitized to Blo t. Co-sensitization was found in 70.14% of patients to these three mites. Almost all Blo t-sensitized patients were sensitized to Der p (1056/1071, 98.60%) or Der f (1058/1071, 98.79%), and only seven patients were sensitized solely to Blo t. In contrast, 82.50% of Der p-sensitized and 82.79% of Der f-sensitized patients were sensitized to Blo t.

#### 3.2 Characteristics of the degree of co-sensitization among Blo t, Der p, and Der f

About 50% of Blo t sIgE-negative patients were sensitized to Der p and Der f, whereas of the Blo t-sensitized (sIgE-positive) patients, only about 2% were sIgE-negative to Der p or Der f (Figure 3). Based
on the degree of sensitization, the patients were further classified into three subgroups: mild (class 1-2), moderate (class 3-4), and severe (class 5-6) sensitization. In patients co-sensitized with Blo t and Der p/Der f, as the degree of Blo t sensitization increased, the percentage of those who were severely sensitized to Der p/Der f increased significantly (P < .001; Figure 3A,B). With respect to Der p and Der f (Figure 3C), the sIgE-negative, mild sensitization, moderate sensitization, and severe sensitization consistency rates were 93.09% (202/219), 76.87% (113/143), 74.84% (348/416), and 92.37% (617/719), respectively.

3.3 | Spearman correlation among the sIgE level for Blo t, Der p, and Der f

The results of the spearman correlation analysis are shown in Figure 4. We found that sensitization to Der p was highly correlated with that of Der f (r = .9487, P < .001), while the sensitization of Blo t was moderately correlated with that of Der p and Der f, with correlation coefficients r of .6998 (P < .001) and .6782 (P < .001), respectively.

3.4 | Results of sIgE inhibition assays

Specific IgE inhibition assay was performed among Blo t, Der p, and Der f with 21 individual sera samples from patients co-sensitized with mites, of whom the sIgE concentration is shown in Table 1. Table S1 presents the inhibition rate of IgE against the tested mites after inhibition with Blo t, Der p, and Der f extracts, respectively. Results showed that all the mite extracts almost completely inhibited their serum sIgE (with the median inhibition rate of >95%). Der p and Der f inhibited each other with a rate >44%, whereas the mutual inhibition rate between Blo t and the two Dermatophagoides species was ranged from 1% to 99% (Table S1). Results showed that there were significant differences in the inhibition rate of IgE binding to mites with different as well as same inhibitor, except for the inhibition rate of Der f inhibiting Der p and Der f inhibiting Blo t, as well as the inhibition rate of Blo t inhibiting Der p and Blo t inhibiting Der f (Figure 5).

The mutual inhibition ability of the mites was compared by Mann-Whitney U test. Figure 6A shows that the inhibition between Der p and Blo t, as well as that between Der f and Blo t, was significantly different (P = .004 and P = .03, respectively). Then, we divided patients into three groups according to the relative class of sIgE. Significant differences were found in the patient groups C_{Blo t} < C_{Der p} and C_{Blo t} < C_{Der f} (Figure 6B,C). Those with a positive inhibition rate (Blo t extract inhibited IgE binding to Der p or Der f) were lower than the reverse one (Der p or Der f extract inhibited IgE binding to Blo t; P < .001). Four sera samples in group C_{Blo t} > C_{Der p} and three sera samples in C_{Blo t} > C_{Der f} had decreased reverse inhibition rates, but were not significantly different. In group C_{Blo t} = C_{Der p} the reverse inhibition rates were higher than the positive ones in all sera samples but were not statistically significant (Figure 6B). However, in group C_{Blo t} = C_{Der f} the mutual inhibition in the sera was random, and there was no significant difference in (Figure 6C).

In addition, eight sera samples from patients with Der p and Der f sIgE-positive but Blo t sIgE-negative were selected for the sIgE inhibition assay, and the characteristics and inhibition results of these patients are shown in Table S2.

4 | DISCUSSION

In this study, the prevalence of Blo t, Der p, and Der f was measured in 1497 patients those who were at least sensitized to one mite, so the sensitization rates of them in our study were higher than that of a previous SPT research in this area. Among the mite-sensitized subjects, >98% of patients were co-sensitized to at least two mites (Blo t, Der p, and Der f), and more than 70% of patients were sensitized to all three mites. These data indicate that not only Der p and Der f but also Blo t contributes to the allergic response in Guangzhou. Blo t-sensitized patients, and those co-sensitized to Der p or Der f, might have multiple sensitizations and/or cross-reaction.

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**FIGURE 4** Spearman correlation among the sIgE level for Blo t, Der p, and Der f. Correlation analyses between non-parametric data were performed using Spearman’s tests, with the correlation coefficients presented as “r_s.” Blo t, *Blomia tropicalis*; Der f, *Dermatophagoides farinae*; Der p, *Dermatophagoides pteronyssinus*; sIgE, specific IgE.
Within the correlation analysis of sIgE, there was a highly positive correlation of sIgE between Der p and Der f, and a moderate positive correlation of sIgE between Blo t and Der p and between Blo t and Der f, which fit well with the findings of Kobi Sade et al. Moreover, our results showed that as the severity of Blo t sensitization rose, the percentage of patients who were co-sensitized at a severe level to Der p/Der f also increased, indicating that cross-reaction was more likely to account for Blo t sensitization.

Zheng et al. reported the indoor allergen levels in Guangzhou city and showed that the median values of Der p 1 and Der f 1 in dust samples from living rooms and bedrooms were 0.015-0.33 μg/g and 0.12-5.4 μg/g, respectively. They also showed that 38% of dust samples from bedding had levels of HDM allergen at or above 10 μg/g, while the median level of Blo t was below the lower limit of detection, with only 3.5%-27% of samples above the lower limit of detection. The absence of Blo t allergen also supported to our hypothesis that the high prevalence of Blo t along with co-sensitization might be because of IgE cross-reactivity toward Dermatophagoides species.

To distinguish between the true sensitization and cross-reactivity of the three mites, 21 sera samples were selected for sIgE inhibition. The inhibition between Der p and Der f displayed a large degree of IgE-mediated cross-reactivity, in agreement with other studies, while the inhibition rate between Blo t and these two mites ranged from 1% to 99%, which was inconsistent with previous studies. Perhaps, our study’s large sample size and greater variety of sIgE classes to Der p and Blo t could account for this difference.

To explain the wide inhibition rate, there was a significant difference between the ability of Der p and Der f to inhibit IgE binding to Blo t. Der p and Der f could inhibit IgE binding to Blo t better than...
Blo t inhibit Der p or Der f in patients of Group C_{Blo t} < C_{Der p} and C_{Blo t} < C_{Der f}. The binding of Der p to sera sIgE could only be inhibited by Blo t with maximum inhibition rate of 60%, while Blo t sIgE could be inhibited by Der p from 50% to 99%. This indicates that Blo t sensitization in these patients is more likely not because of true sensitization but cross-reaction with Der p and Der f.

In contrast, for patients in groups C_{Blo t} > C_{Der p} and C_{Blo t} > C_{Der f}, no significant difference was revealed in the mutual inhibition between Blo t and Der p, as well as Blo t and Der f. It seems that there is a subset of people sensitized to mites but with the class of sIgE to Blo t higher than that of Der p or Der f who are sensitized to Blo t that is not explained by cross-reactivity to Der p or Der f exposure. In these mite-sensitized patients, cross-reactivity between Blo t and the other two mites is limited.

Furthermore, we have also selected eight patients who are Der p and Der f sIgE-positive (Class of sIgE level ≥3) and Blo t sIgE-negative patients to perform sIgE inhibition assay. Consistent with the results of Kim et al., our study showed that the binding of IgE to Der p and Der f was not inhibited by Blo t, indicating that cross-reactivity between Der p/Der f and Blo t did not contribute to the sensitization of Der p or Der f in these patients. Thus, the positivity of Der p and Der f in these patients was because of true sensitization.

The advantages of our study are as follows. On one hand, a large number of allergic patients suspected to be sensitized to mites were selected in our study, offering valuable information about the IgE co-sensitization to the three mites. On the other hand, the sera for sIgE inhibition assay (ImmunoCAP) were randomly selected from patients with different degree of sensitization to Blo t, Der p, and Der f, which made our analysis of cross-reactivity more comprehensive. The limitation of this study was that the sera for the sIgE inhibition assay were insufficient because of the small number of patients in Group C_{Blo t} > C_{Der p} in Guangzhou city. More individual sera are needed in subsequent sIgE inhibition assays.

5 | CONCLUSIONS

In conclusion, sensitization to Blo t commonly coexists with sensitization to two Dermatophagoides species (Der p and Der f) and the IgE sensitization among them was moderately correlated. As for patients co-sensitized to mites, those who with the lower sIgE levels to Blo t are more likely to be caused by cross-reaction, while the higher sIgE level in Blo t-sensitized patients may be due to both of cross-reaction and true sensitization. S IgE inhibition assay could be used to identify cross-reaction or true sensitization. Further research recognizing the clear cross-reactive components of Blo t and two Dermatophagoides species could be performed using recombinant allergenic components.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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