

Immune Response to *Blomia kulagini* and *Dermatophagoides pteronyssinus* in Sweden and Brazil

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Abstract. The immune responses to the non-pyroglyphid mite *Blomia kulagini* and the pyroglyphid mite *Dermatophagoides pteronyssinus* were compared in 440 Swedish farmers and 34 allergic subjects from Brazil using the radioallergosorbent test (RAST). Both *B. kulagini* and *D. pteronyssinus* were shown to be a common cause of sensitization, particularly in Brazil. The highest RAST scores were found against *D. pteronyssinus* in sera from both countries. Allergenic cross-reactivity between the two mites was studied by the RAST inhibition technique. Both *B. kulagini* and *D. pteronyssinus* possess their own unique allergens as well as allergens in common. Thus, besides mites belonging to the *Dermatophagoides* genus, other pyroglyphid and non-pyroglyphid mites in different countries should be taken into consideration before starting hyposensitization treatments.

Introduction

Twenty years ago the mites belonging to the genus *Dermatophagoides* were shown to be the major source of allergen in house dust by Voorhorst et al. [1]. In fact, *Dermatophagoides pteronyssinus* is the predominant mite species encountered in houses in northern Europe and *Dermatophagoides farinae* in North America [2]. Several studies have indicated that sensitization to non-pyroglyphid mites is an occupational hazard in farming environments and for people living in damp houses where non-pyroglyphid mites may increase more rapidly and eventually constitute a higher proportion of the total mite population than the Pyroglyphidae family [3]. Other subsequent studies [4-8] have shown that non-pyroglyphid mites compose up to 24% of the acarofauna of house dust.

Besides the most important storage mites, *Lepidoglyphus destructor*, *Acarus siro*, *Glycyphagus domesticus*, and *Tyrophagus putrescentiae*, the less known non-pyroglyphid mite genus *Blomia*, which includes *Blomia tropicalis*, *Blomia freemani*, and *Blomia kulagini*, gives rise to allergic manifestations. Sensitization after exposure to these mites has been reported from Japan [9] and South America [10]. We have previously

studied the allergenic properties of *B. kulagini*, dominating in Brazil, and *L. destructor*, the most common storage mite in Sweden [van Hage-Hamsten et al., unpubl. data], among subjects from Brazil and Sweden. The two populations showed different radioallergosorbent test (RAST) inhibition [11] patterns when allergenic cross-reactivity between these two mite species was studied. This probably depends on differences in sensitization. In this study we shall report data on IgE-mediated response to the storage mite *B. kulagini* and the house dust mite *D. pteronyssinus* in individuals from Sweden and Brazil. Studies using inhibition of the RAST have also been performed to compare the allergenicity between the two mite species.

Subjects and Methods

Sera from 440 farmers from the Swedish island of Gotland ('Swedish farmers') were examined with RAST for IgE antibodies against *B. kulagini* and *D. pteronyssinus*. These farmers had previously participated in an epidemiological survey of respiratory symptoms [12].

Thirty-four sera from atopic patients, aged 5-62 years, with asthma and/or rhinoconjunctivitis attending the Allergy Clinic at

Hospital Das Clinicas in São Paulo ('Brazilian patients') were studied together with the Swedish sera. These patients had a history indicative of allergy to house dust and a positive skin prick test to *D. pteronyssinus* and *D. farinae* (Pharmalgen, 100,000 BU; Pharmacia Diagnostics, Uppsala, Sweden).

Based on the RAST results, the allergenic cross-reactivity between *B. kulagini* and *D. pteronyssinus* could be evaluated. Sera from 6 Swedish farmers and 6 patients from Brazil who were RAST positive to both *B. kulagini* and *D. pteronyssinus* were inhibited with extracts prepared from these two mite species. Sera from 4 more farmers and 2 more subjects from Brazil were inhibited with one extract (homologous system) only, and RAST were then performed against appropriate allergen discs. To demonstrate the presence of unique allergen(s) in *B. kulagini* in comparison with *D. pteronyssinus*, five sera from Sweden and three sera from Brazil, which were either RAST positive to *B. kulagini* and negative to *D. pteronyssinus*, or the reverse, were inhibited with both *B. kulagini* and *D. pteronyssinus* extracts. All serum samples had been kept frozen at -20°C until analyzed.

Allergen Preparations

Extracts, 1/10 weight/volume (w/vol), were prepared from *B. kulagini* and *D. pteronyssinus* in 0.15 M NaCl containing 0.02% NaN₃, using raw material obtained from Allergon (Ängelholm, Sweden) and Pharmacia Diagnostics (Uppsala, Sweden), respectively.

Determination of IgE Antibody

Specific IgE measurements using RAST, as described by Johansson et al. [13], were performed against *D. pteronyssinus* and *B. kulagini*. The counts per minute (cpm) were related to those of the Phadebas RAST reference system (Pharmacia Diagnostics) and the results expressed in Phadebas RAST units (PRU) per milliliter. Allergen discs with *D. pteronyssinus* (dl) were obtained from Pharmacia Diagnostics, and *B. kulagini* discs were prepared by coupling 0.5 ml of a 1/10 w/vol extract to 100 cyanogen bromide-activated paper discs [14]. The discs gave no non-specific uptake of labelled anti-IgE when tested against non-atopic serum samples with IgE levels of 20–4,000 kU/ml. The discs did not contain significant amounts of wheat allergen, a potent inhalation allergen commonly used in mite growth media. All sera ≥ 0.20 PRU were retested in duplicate. A positive result was defined as ≥ 0.35 PRU/ml.

RAST Inhibition

Mite allergen extracts were mixed at various dilutions with serum from subjects with IgE antibodies against storage and/or house dust mites [15]. The serum samples were first diluted 1/1–1/10 to give approximately 5,000 cpm in RAST. The level of free IgE antibodies was then determined by RAST. The results were expressed as the percentage of the cpm value of the positive serum dilution after inhibition with allergen extract diluent, according to the following formula:

$$\% \text{ inhibition} = 100 - \frac{\text{cpm after inhibition with extract}}{\text{cpm after inhibition with diluent}} \times 100.$$

A background value (buffer value) averaging 200 cpm was always subtracted from the actual count rate. The potency of the extracts was compared at 50% RAST inhibition. The inhibitory capacity

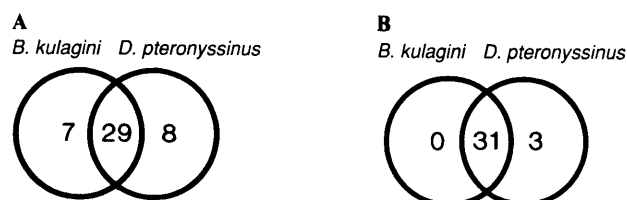


Fig. 1. Distribution of positive (≥ 0.35 PRU/ml) tests against *B. kulagini* and *D. pteronyssinus* in sera from Sweden (A) and Brazil (B).

was expressed in arbitrary units (U). The highest concentration of an extract used was assigned the value 1,000 U/ml. The dilution of the 1,000-U/ml solution of each extract was selected so that 50% RAST inhibition of the homologous system (e.g., the *B. kulagini* extract inhibiting the positive RAST to *B. kulagini* allergens discs) was obtained at an extract concentration in the order of 0.1–10 U/ml. For the *B. kulagini* and the *D. pteronyssinus* extracts the concentration of 1,000 U/ml represents 1/10 w/vol. A final concentration of 500 U/ml corresponds to an extract concentration of 1,000 U/ml.

Statistical Analysis

To estimate the strength of the allergenic relationships between the two mite species, Spearman rank correlation coefficients between positive RAST scores (positive to at least 1 of the 2 species) were calculated. Estimates of correlation coefficients were tested for significant departure from zero. For all tests $p < 0.05$ was considered to be significant.

Results

Distribution of Positive RAST

After analyzing the 440 Swedish farmers, 44 proved to be RAST positive to at least 1 of the 2 mite species. Twenty-nine of the 44 (65.9%) were RAST positive to both *B. kulagini* and *D. pteronyssinus* (fig. 1A), whereas 36 of 44 (81.8%) had IgE antibodies to *B. kulagini* and 37 of 44 (84.1%) to *D. pteronyssinus*. IgE antibodies solely to *B. kulagini* and *D. pteronyssinus* were found in 7 (15.9%) and 8 (18.2%) of the farmers, respectively.

All patients from Brazil, 34 of 34, had IgE antibodies to *D. pteronyssinus* and 31 of 34 to *B. kulagini* (fig. 1B).

RAST Correlation Studies

No significant RAST correlation was found between *B. kulagini* and *D. pteronyssinus* in the Swedish farmers (0.22, n.s.; fig. 2A) in contrast to the Brazilian subjects (0.58, $p < 0.01$; fig. 2B). Both the Swedish farmers and the Brazilian subjects generally had a

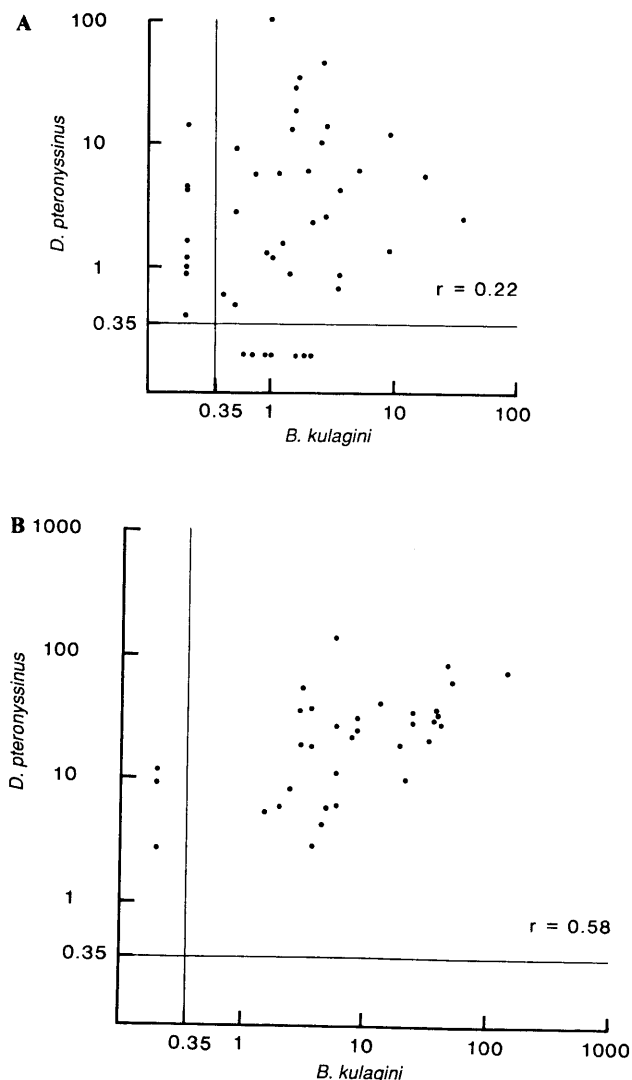


Fig. 2. RAST scores to *B. kulagini* and *D. pteronyssinus*. The correlation coefficient was calculated among all Swedish farmers (A) and the patients from Brazil (B) with a positive RAST (≥ 0.35 PRU/ml) to at least one of the two mites.

higher IgE response to *D. pteronyssinus* than to *B. kulagini*.

RAST Inhibition

The inhibitory capacity of the *B. kulagini* and the *D. pteronyssinus* extracts on twelve sera which were RAST positive to both mite species is shown in figures 3 and 4. In only one Brazilian serum the same amount of *B. kulagini* extract was able to achieve a 50% inhibition of both the homologous system (a positive RAST to *B. kulagini* allergen disc) and the *D. pteronyssinus* system. However, when 10–1,000 times more

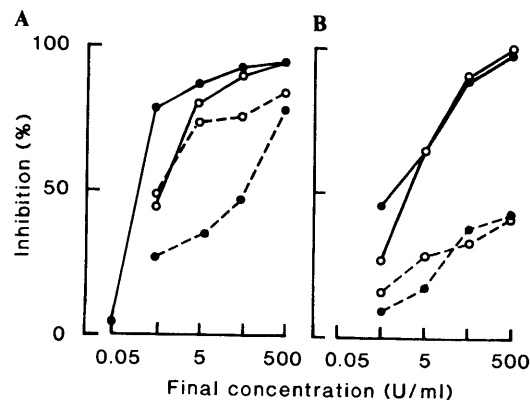


Fig. 3. The inhibiting effect of *B. kulagini* extract on a positive RAST to discs coated with *B. kulagini* (●—●) and *D. pteronyssinus* (●---●) and the inhibiting effect of *D. pteronyssinus* extract on a positive RAST to discs coated with *B. kulagini* (○---○) and *D. pteronyssinus* (○—○) on sera from Sweden (A) Brazil (B).

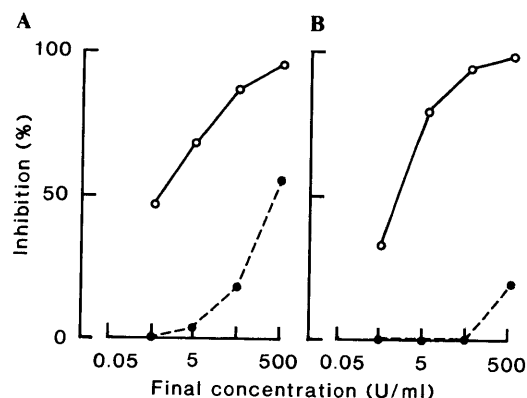


Fig. 4. The inhibiting effect of extracts of *B. kulagini* (●---●) and *D. pteronyssinus* (○—○) on sera from Sweden (A) and Brazil (B) which are RAST positive to *D. pteronyssinus* and negative to *B. kulagini*. The discs were coated with *D. pteronyssinus*.

B. kulagini extract than required in the homologous system was used, a 50% inhibition of the *D. pteronyssinus* system was obtained in 5 out of 6 Swedish sera (fig. 3A) and in 1 Brazilian serum. Furthermore, in 1 Swedish serum and 4 Brazilian (fig. 3B) sera, a 50% inhibition of the *D. pteronyssinus* system was not obtained, even at the highest concentration of the *B. kulagini* extract added (1,000 U/ml). Similar results, not illustrated here, were found with the *D. pteronyssinus* extract. However, the same amount of *D. pteronyssinus* extract could achieve a 50% inhibition of both the *D. pteronyssinus* and the *B. kulagini* systems in

one third of the Swedish (fig. 3A) and the Brazilian sera.

In a further attempt to understand the allergenic relationship between *B. kulagini* and *D. pteronyssinus*, we analyzed 3 Brazilian subjects and 3 Swedish farmers who were negative in RAST to *B. kulagini* and positive to *D. pteronyssinus*. In comparison with the homologous system, even 100–1,000 times more *B. kulagini* extract could not inhibit the *D. pteronyssinus* system more than 60% in 3 Swedish sera (fig. 4A) and 40% or less in 3 Brazilian sera (fig. 4B). The *D. pteronyssinus* extract, on the other hand, could produce a 50% inhibition of the *D. pteronyssinus* system when 1,000 U/ml was added to 1 of 2 Swedish sera tested which were positive in RAST to *B. kulagini* and negative to *D. pteronyssinus*.

In 4 Swedish and 2 Brazilian sera one concentration only of the extracts of *D. pteronyssinus* and *B. kulagini* was added to achieve a 50% inhibition of the homologous system. The results supported the findings of our detailed dose-related RAST inhibition study.

Discussion

The increasing interest in allergy to non-pyroglyphid mites has prompted several studies on IgE-mediated sensitization to these mite species in both farming [12, 16, 17] and non-farming populations [4, 6, 18–20]. We have earlier reported that storage mites seem to be the most important allergen among farmers. The prevalence of allergy to these mite species was as high as 6.2% [12] in a Swedish farming population, whereas sensitization to pollens, animal danders, and moulds was rare [21], although farmers are daily exposed to such common potent allergens. The most important storage mite species with respect to positive RAST results among farmers is *L. destructor* [12], according to a mite inventory of Swedish barn dust [van Hage-Hamsten et al., in preparation]. This mite species causes symptoms from both upper and lower airways and eyes. Bronchial provocation studies have confirmed that *L. destructor* provokes asthma in sensitized subjects [22]. Not only farmers, but also subjects in other occupations, like grain elevator workers [23] or bakers [24], can develop IgE-mediated sensitization to non-pyroglyphid mites.

Blomia is a common mite in house dust in tropical and subtropical regions [25, 26]. Large infestations of

Blomia in house dust have been reported from Hong Kong [4] and Japan [9]. Miyamoto et al. [9] have also reported sensitization to this mite species with the use of skin tests. Recently, Hurtado and Parini [10] from Venezuela showed that *B. tropicalis* was present in 96% of bedding dust samples in Caracas. This particular mite is found in 30% of dust samples in Florida, USA, as well [Fernandez-Caldas, personal commun.]. A similar investigation of homes in the surroundings of São Paulo revealed that *Blomia* species are present in house dust samples to the same extent as in Florida [Croce, personal commun.]. Thus, *Blomia* might be an important sensitizer in different parts of the world.

The non-pyroglyphid mites *B. kulagini* and *L. destructor* belong to the same family, the Glycyphagidae, whereas *D. pteronyssinus* belongs to the Pyroglyphidae. The RAST correlation analyses between *B. kulagini* and *D. pteronyssinus*, based on both Swedish and Brazilian sera, showed that the highest IgE responses were found against *D. pteronyssinus*. The presence of unique allergens in both mite species is supported by the existence of subjects with positive RAST values against either *B. kulagini* or *D. pteronyssinus*. A similar low correlation coefficient was found between the RAST scores against *D. pteronyssinus* and *B. kulagini*, as we have previously shown for *D. pteronyssinus* and *L. destructor* ($r = 0.18$), in sera from Swedish farmers [15]. Likewise, the fairly high correlation coefficient between *D. pteronyssinus* and *B. kulagini* in the Brazilian sera was close to the one earlier reported between *L. destructor* and *B. kulagini* ($r = 0.56$) [van Hage-Hamsten et al., unpubl. data]. These results with respect to *Blomia* and *Dermatophagoides* primarily indicate that the latter mite species is the main sensitizer in both countries, whereas in a Swedish farming population *L. destructor* is dominating compared with *D. pteronyssinus*.

The RAST inhibition data confirm that *B. kulagini* and *D. pteronyssinus* possess their own unique allergens as well as common allergenic determinants. However, *D. pteronyssinus* is lacking *B. kulagini* unique allergens, since at least 100–1,000 times more *D. pteronyssinus* extract was needed to achieve a 50% inhibition of the *B. kulagini* system in half of the Swedish and Brazilian sera which were RAST positive to *D. pteronyssinus* as well as to *B. kulagini*. The reverse was found with the *B. kulagini* extract. These findings are in contrast to our earlier studies on cross-reactivity between *B. kulagini* and *L. destructor* where

the sera from Brazil and Sweden showed a different inhibition pattern. A similar amount of *L. destructor* extract was able to inhibit the homologous system and the *B. kulagini* system in Swedish but not in Brazilian sera. This most likely depends on the fact that *B. kulagini* is the main sensitizer in Brazil, whereas *L. destructor* is dominating in Sweden [van Hage-Hamsten et al., unpubl. data].

Regarding allergenic cross-reactivity between non-pyroglyphid and pyroglyphid mites, we reported in 1987 that these two families do not cross-react. These data were later confirmed in vivo by bronchial challenges [22]. However, Miyamoto et al. [9] have reported some degree of cross-reactivity between *D. farinae* and *Blomia* species. Although *B. kulagini* and *D. pteronyssinus* cross-reacted to some extent in this study, most sera required over 100–1,000 times more of the respective extract to obtain a 50% inhibition compared to the amount needed in the homologous system. Accordingly, using our sera, the RAST inhibition studies suggest that *D. pteronyssinus* possesses some allergenic components inherent in *B. kulagini*, while *B. kulagini* seems to be deficient in some allergenic determinants of *D. pteronyssinus*.

Why the *B. kulagini* extract partially inhibited the *D. pteronyssinus* system in some instances, despite sera from both Sweden and Brazil being negative in RAST, is not evident. One reason may be inadequate concentration of the inhibiting allergenic component on the *B. kulagini* disc. We recently found the same results when studying the cross-reactivity between *B. kulagini* and *L. destructor* [van Hage-Hamsten et al., unpubl. data] as well as between *D. pteronyssinus* and the less known house dust mite *Euroglyphus maynei* [27]. Thus, it is possible that in the case of minor allergens of low concentration, sufficient amounts are not always obtained on the RAST disc using the present procedures.

Although mites belonging to the *Dermatogoides* genus dominate in the acarofauna, non-pyroglyphid mites are certainly of clinical importance. Unfortunately, many physicians are so far only considering mites of the *Dermatophagoides* genus as a possible source of the allergic symptoms. In order to ensure good results from hyposensitization treatment, however, they should consider which other mite species might cause IgE-mediated sensitization in that particular country. Further characterization studies of different mite species are in progress to clarify the importance of related allergens.

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