

Original article

Allergenic composition of the mite *Suidasia medanensis* and cross-reactivity with *Blomia tropicalis*

Background: Mites of the genus *Suidasia* are commonly found in house dust and may play an allergenic role in exposed populations. However, the allergenic potential and clinical impact of this genus has not been well established. The main objective of this project was to evaluate the allergenic role of the mite *Suidasia medanensis*.

Methods: An extract of *S. medanensis* was prepared and the allergen composition determined by immunoblot. Specific IgE antibody levels to *S. medanensis* and *Blomia tropicalis* were evaluated by radioallergosorbent (RAST) in the sera of 97 allergic asthmatic patients and 50 nonallergic subjects. Cross-reactivity between *S. medanensis* and the mite species *B. tropicalis* and *Dermatophagoides farinae* was investigated by RAST and immunoblot inhibitions.

Results: Seventy-one asthmatic patients sera (73.2%) had positive IgE reactivity to *S. medanensis*; 14 allergens with molecular weights ranging from 7.5 to 105 kDa were detected. The most frequently detected had molecular weights of 30–31 (54.8%), 24.5 (42%), 21 (38.7%), 47 (35%) and 58 kDa (35.5%). *Blomia tropicalis* extract inhibited IgE binding to nine of these identified allergens. Four *B. tropicalis* allergens were inhibited by *S. medanensis* extract. RAST inhibition results demonstrated a high degree of inhibition by *B. tropicalis* (87.2%) and *D. farinae* (90.9%) than by *S. medanensis* (32%).

Conclusions: Sensitization to *S. medanensis* is common in asthmatic allergy patients in Cartagena. An important degree of cross-reactivity was established between *S. medanensis* and *B. tropicalis*, and *D. farinae*.

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Mite allergens are a common cause of allergic respiratory diseases in many regions of the world. Pyroglyphid mites, such as *Dermatophagoides pteronyssinus* and *D. farinae* have a worldwide distribution and have been identified as important sources of house dust derived allergens and inducers of allergic respiratory diseases (1, 2). In addition, the mites *Blomia tropicalis*, *Lepidoglyphus destructor*, *Acarus siro* and *Tirophagus putrescentiae* are also important sources of allergens in house dust and their allergenic role has been established (2–7). Other mites, such as those belonging to the genus *Suidasia*, have been found in house dust and could play an allergenic role in some populations (8, 9). However, the allergenic potential and clinical impact of this genus has not been well studied.

Suidasia spp. are mites belonging to the superfamily Acaroidea (Table 1) common in nests of vertebrates and insects and in stored food products (10, 11). The species *S. medanensis*, *S. reticulata*, *S. pontifica* (probably a synonym for *S. medanensis*) and *S. nesbitii* have been isolated from house dust collected in several tropical regions (8, 9, 12, 13). The allergenic role of genus *Suidasia* and its cross-reactivity with other mite species have been

suggested. *Suidasia medanensis* and *S. nesbitii* have been detected in house dust samples collected in urban areas of Puerto Rico (8). In Venezuela *Suidasia* spp. have been associated with cases of anaphylaxis induced by ingestion of wheat contaminated with mites (9). Different species belonging to genus *Suidasia* have been also found in mattress and house dust samples from Malaysia and Colombia, countries where mite allergens play an important role in asthma (12, 13).

Blomia tropicalis and *D. pteronyssinus* are the most common mite species in Cartagena, Colombia. However, other genera such as *Suidasia* are also present in this tropical city (13). The clinical relevance of *S. medanensis* in an asthmatic population of Cartagena was suggested by the fact that a high percentage of the asthmatic patients evaluated were sensitized to this mite species (14). Skin test and radioallergosorbent (RAST) results showed that the frequency of sensitization to several mite species is high in the asthmatic population of Cartagena (15, 16). This finding may be explained by exposure to different mite species involving species-specific allergens or by cross-reactivity allergens (3, 17–20).

Table 1. Taxonomic position of *Suidasia medanensis* and other house dust mites

<u>Superfamily</u>	<u>Family</u>	<u>Subfamily</u>	<u>Genus</u>	<u>Species</u>
Acaroidea	<i>Suidasiidae</i>		<i>Suidasia</i>	<i>S. medanensis</i> , <i>S. reticulata</i> <i>S. nesbitti</i>
	<i>Acaridae</i>		<i>Acarus</i>	<i>A. siro</i>
	<i>Lardoglyphidae</i>		<i>Aleuroglyphus</i>	<i>A. ovatus</i>
	<i>Glycacaridae</i>		<i>Tyrophagus</i>	<i>T. putrescentiae</i>
Glycyphagoidea	<i>Echimyopodidae</i>		<i>Blomia</i>	<i>B. tropicalis</i> , <i>B. thori</i> , <i>B. tjiaboda</i> <i>B. kulagini</i> , <i>B. freemani</i>
	<i>Glycyphagidae</i>	<i>Glycyphaginae</i>	<i>Glycyphagus</i>	<i>G. domesticus</i>
			<i>Lepidoglyphus</i>	<i>L. destructor</i>
	<i>Chortoglyphidae</i>		<i>Chortoglyphus</i>	<i>C. arcuatus</i>
Pyroglyphoidea	<i>Pyroglyphidae</i>	<i>Pyroglyphinae</i>	<i>Euroglypus</i>	<i>E. maynei</i>
		<i>Dermatophagoidinae</i>	<i>Dermatophagoides</i>	<i>D. pteronyssinus</i> , <i>D. farinae</i> <i>D. microceras</i> , <i>D. siboney</i>

Suidasia medanensis has been scarcely studied and its role in the process of sensitization of allergic patients remains to be established. In this study we report, for the first time, the allergenic composition *S. medanensis* and its cross-reactivity with *B. tropicalis* and *D. farinae*.

Materials and methods

Patient population and serum samples

Serum samples were obtained from 97 asthmatic patients living in the urban area of Cartagena, who were skin test and RAST positive to *B. tropicalis* and *D. pteronyssinus* extracts and were not treated with immunotherapy. Sera from 50 healthy volunteers with no history of allergy and negative skin test and RAST to mite allergens were used as negative controls. The asthmatics and controls subjects belong to a population selected in previous studies on epidemiology of respiratory allergies (15, 21). All serum samples were stored at -70°C until used. A serum pool, made by mixing equal volumes of sera from eight asthmatics with levels of specific IgE to *S. medanensis*, *B. tropicalis* and *D. farinae* higher than 5% of total counts bound (% TCB), was used in RAST and immunoblot inhibitions.

Allergenic extracts

Suidasia medanensis mites were isolated from house dust samples from homes of allergic patients living in Cartagena and cultured in the Mites Laboratory of The Institute of Immunological Research using Tetramin® fish food at 26°C and 86% relative humidity. Adult mite bodies were separated from the food medium with a modified Tullgren apparatus. Mite bodies were defatted for 4 h with anhydrous ether in a Soxhlet extractor and dried at room temperature (RT). Defatted mites were extracted (1 : 20 w/v) in 0.1 ml/l of ammonium bicarbonate for 24 h at 4°C . The extract was clarified by centrifugation at 9000 g and dialyzed overnight (ON) at 4°C against deionized water with a 3500 molecular weight cutoff membrane (Spectrum Medical Industries, Inc., Los Angeles, CA, USA). The dialyzed extract was centrifuged and filtered with 0.22 μm membrane, and stored at -20°C . Protein concentration was determined by the Bradford method. *Dermatophagoides farinae*

extract was prepared from mite bodies, following the same procedure. *Blomia tropicalis* extract was prepared as previously described (3).

Specific IgE determination

Specific IgE to mite allergenic extract was determined by RAST. Microtiter-plate wells were coated with 100 μl of extract diluted 1 : 50 in 0.2 mol/l sodium carbonate/bicarbonate buffer, pH 9.2. The plates were incubate ON at RT and washed with PBS/0.1% Tween 20. One hundred microliters of serum was added to wells, incubate ON, washed three times and incubated with 100 μl of ^{125}I -labeled horse anti human IgE (Kallestad Diagnosis Inc., Chaska, MN, USA). After ON incubation, the plates were washed and each well counted in a gamma counter. All samples were analyzed in duplicate and the results expressed as %TCB. RAST was considered positive when serum bound more than 1% of TCB.

RAST inhibition

To evaluate the degree of cross-reactivity between extracts of *S. medanensis* and *B. tropicalis* and *D. farinae*, inhibition assay was performed as previously described (3). Briefly, specific IgE determination was performed with serial dilutions of the serum pool to establish the optimal dilution for the inhibition. Serum pool diluted 1 : 24; 1 : 32 and 1 : 8 was used when the inhibitors were *S. medanensis*, *B. tropicalis* and *D. farinae*, respectively. Fifty microliters of the pool were adsorbed with 50 μl of serial concentrations of the extracts. After adsorption, the mix was loaded into the wells coated with allergenic extract and incubated ON at RT. The reaction was developed as for specific IgE determinations. The percentage of inhibition was calculated as follows: Percent inhibition = (c.p.m. without inhibitor – c.p.m. with inhibitor/c.p.m. without inhibitor) $\times$ 100.

Immunoblotting and immunoblot inhibition

SDS-PAGE was performed in a 15% gel using 50 μg extract per lane in a Mini-Protein II^R system (Bio-Rad, Richmond, CA, USA), under reducing conditions. Then the proteins were transferred to a

nitrocellulose (NC) membrane (Trans-Blot NC; Bio-Rad). The membranes were blocked with PBS/3% BSA for 3 h, washed and cut into strips (3 mm). Each strip was incubated for 16 h with 2 ml of serum diluted 1 : 5 in PBS. After washing the strips were incubated with ^{125} I-labeled anti-human IgE and the allergen fractions detected by autoradiography. The molecular weight of each mite allergen was determined by comparing its relative mobility with that of low molecular weight standard protein (Bio-Rad).

For immunoblot inhibition, the serum pool was pre-incubated for 2 h at RT with 100 μ g of each mite extract. The final dilution of the serum pool was 1 : 10. The mixtures were incubated with blotted mite proteins on NC strips and the allergens visualized following the same protocol as immunoblotting.

Results

Specific IgE levels

Seventy-one patients (73.2%) were positive to *S. medanensis*, with RAST values ranging from 1.0 to 53% TCB (geometric mean 2.37). Eighty patients (83.3%) were positive to *B. tropicalis*, ranging from 1.0 to 56.3% TCB (geometric mean 6.16). In the control group, five patients (10%) were RAST positive to *S. medanensis* and none to *B. tropicalis* ($P < 0.001$, Mann-Whitney test) (Fig. 1). Sixty-seven sera (69%) were positive to both *S. medanensis* and *B. tropicalis* extracts. Positive RAST to *D. farinae* and *D. pteronyssinus* was demonstrated in three sera, which were positive to *S. medanensis* but not to *B. tropicalis* and thirteen sera, which were positive for *B. tropicalis* but not to *S. medanensis*.

Allergens of *S. medanensis*

Thirty-one sera were selected to identify IgE-binding components in the *S. medanensis* extract. Figure 2 shows data obtained with some of the sera tested. Fourteen IgE-binding components, with molecular weight ranging

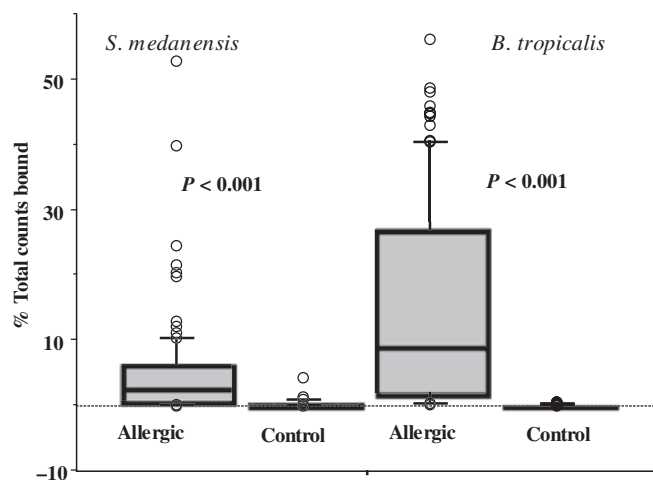


Figure 1. Boxplot view of RAST results to *S. medanensis* and *B. tropicalis* extracts. Distribution of IgE levels in 97 asthmatics and 50 nonallergic subjects.

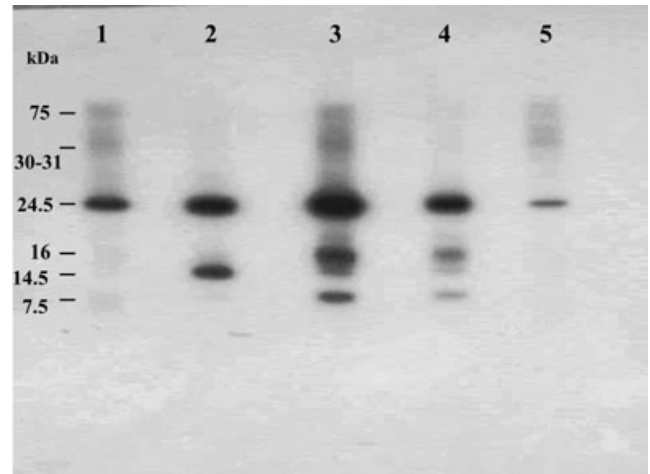


Figure 2. Autoradiography of immunoblot analysis of a *S. medanensis* extract. Lanes 1–5 show pattern of IgE reactivity of five allergic patients.

from 7.5 to 105 kDa, were clearly detected. The most frequently detected allergenic components were: 30–31 kDa (54.8%), 24.5 (42%), 21 (38.7%), 47 (35%) and 58 kDa (35.5%). The remaining allergens (7.5, 14.5, 16, 27.5, 35.5, 41–42, 66, 75 and 105 kDa) were detected in lower frequencies. No bands were detected when the negative serum pool was used. Table 2 shows the allergogram obtained when testing the 31 sera.

Immunoblot inhibition

The ability of *B. tropicalis* extract to inhibit the IgE binding of the serum pool to *S. medanensis* is shown in Fig. 3. *Blomia tropicalis* extract inhibited IgE binding to the 105, 75, 58, 47, 41–42, 35.5, 30–31, 27.5 and 21 kDa bands. When *B. tropicalis* extract was on solid phase, *S. medanensis* extract completely inhibited the IgE binding to the 25 and 28 kDa allergens and partially inhibited the IgE binding to the 18 and 51.5 kDa allergens (Fig. 4). Inhibition of IgE binding to these bands was also observed with *D. farinae* extract.

Absorption of the serum pool with *D. farinae* extract inhibited the IgE binding to 105, 75, 58, 47, 41–42, 35.5, 30–31, 27.5 and 21 kDa bands of *S. medanensis* extract (Fig. 3). Complete inhibition of the IgE binding to the 11.5, 18, 25 and 28 kDa bands and partial inhibition of the 16, and 51.5 kDa bands of *B. tropicalis* was observed (Fig. 4). In immunoblot inhibition using *D. farinae* extract on solid phase, *S. medanensis* and *B. tropicalis* extracts completely inhibited the IgE binding to the 21, 29, 43, 60 and 115 kDa allergens, and partially the 16 kDa allergen (data not shown).

RAST inhibition

Using *S. medanensis* extract on the solid phase, *B. tropicalis* reached 50% inhibition at 0.95 ng/ μ l and 87.2% inhibition

Table 2. Allergens of *S. medanensis* detected by immunoblotting using individual sera from 31 allergic patients

Serum	Molecular weight (kD)														% RAST	
	7.5	14.5	16	21	24.5	27.5	30–31	35.5	41–42	47	58	66	75	105	<i>S. m.</i>	<i>B. t.</i>
CB29			X	X		X	X	X	X		X		X	X	53	40.4
A21			X	X			X			X			X	X	10.3	27.3
CB22				X		X	X	X		X	X		X	X	20.4	43.5
A124				X		X	X	X		X				X	12.14	5.9
A115				X			X	X	X	X			X		11.2	7.4
A104				X			X	X	X	X	X		X	X	10.3	37.9
A167			X	X			X	X	X	X					40	29
A173			X	X	X		X	X		X				X	21.6	17.6
CB35				X		X	X	X	X	X				X	10.4	44.9
CB8				X						X			X	X	13	5.1
CB21				X		X	X	X		X				X	9.5	25
CB6									X		X	X			7.5	38.5
CB33									X		X	X			6.6	5.2
A66											X	X			6.9	46
A53											X	X			5.8	13.3
A162	X	X			X		X								6	16.7
A141					X		X				X	X			7.5	7.1
A99					X	X	X		X		X	X			6.5	48.7
A109						X	X				X	X			7.4	32.4
A151		X			X										5.7	10.5
A138					X										5.6	4.3
A149		X		X	X		X		X			X			6.6	0.5
A188	X	X	X		X										7.8	18.1
A106	X	X	X		X										8.5	16.3
A44					X	X	X								4.4	7.8
A153							X								2.9	44.3
A119	X		X		X										4.4	13.7
A250					X					X	X		X		2.4	40.7
A161		X						X							2.4	1.5
CB36		X													4.1	23.2
A72					X										2.9	1.3
Total (%)	4 (12.9)	7 (22.5)	7 (22.6)	12 (38.7)	13 (42)	8 (25.8)	17 (54.8)	10 (32.2)	9 (29)	11 (35.5)	11 (35.5)	8 (25.8)	7 (22.6)	9 (29)		

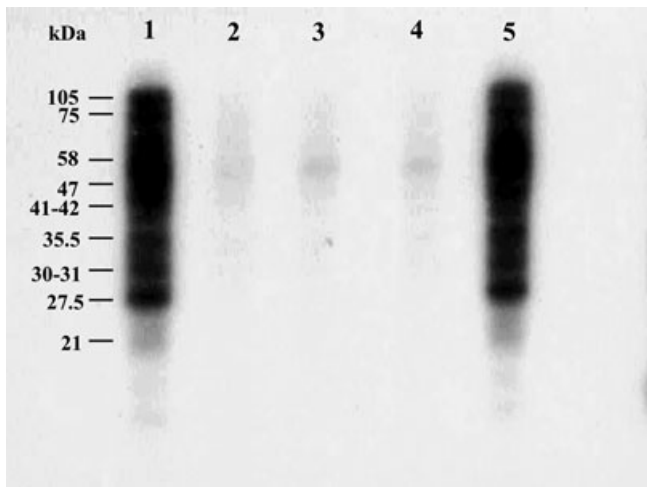


Figure 3. Inhibition of the IgE binding to *S. medanensis* allergens using the serum pool with the following inhibitors: lane 1, no inhibitor; lane 2, *B. tropicalis*; lane 3, *D. farinae*; lane 4, *S. medanensis*; lane 5, BSA.

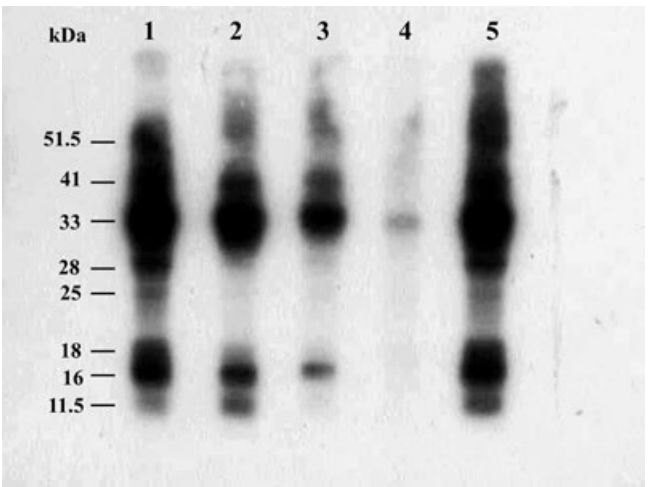


Figure 4. Inhibition of the IgE binding to *B. tropicalis* allergens using the serum pool with the following inhibitors: lane 1, no inhibitor; lane 2, *S. medanensis*; lane 3, *D. farinae*; lane 4, *B. tropicalis*; lane 5, BSA.

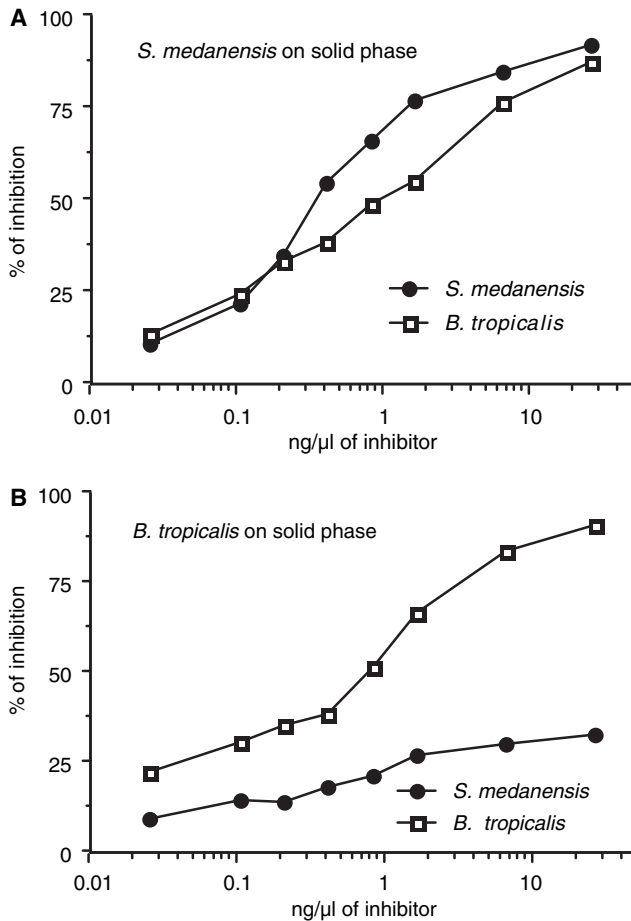


Figure 5. RAST inhibition curve with (A) *S. medanensis* and (B) *B. tropicalis* on solid phase (bound to the microtiter wells). The serum pool was adsorbed with different concentrations of inhibitor allergen: *B. tropicalis* or *S. medanensis* extracts.

at 26 ng/μl, the highest concentration used (Fig. 5A). Using *B. tropicalis* on the solid phase, *S. medanensis* reached 32% of inhibition at 26 ng/μl (Fig. 5B).

When *S. medanensis* extract was on solid phase, *D. farinae* reached 90.9% inhibition, at the highest concentration of inhibitor used (Fig. 6A). When *D. farinae* extract was on solid phase, *S. medanensis* reached 32% inhibition (Fig. 6B). These results suggest that an important degree of cross-reactivity exists between *S. medanensis* and *B. tropicalis*, and between *S. medanensis* and *D. farinae*. However, the pattern of inhibition indicates that the main IgE antibodies are directed against *B. tropicalis* and *D. farinae*.

Discussion

This is the first report describing the allergenic composition of *S. medanensis*. This study documents a high frequency (73.20%) of positive RAST to this mite in

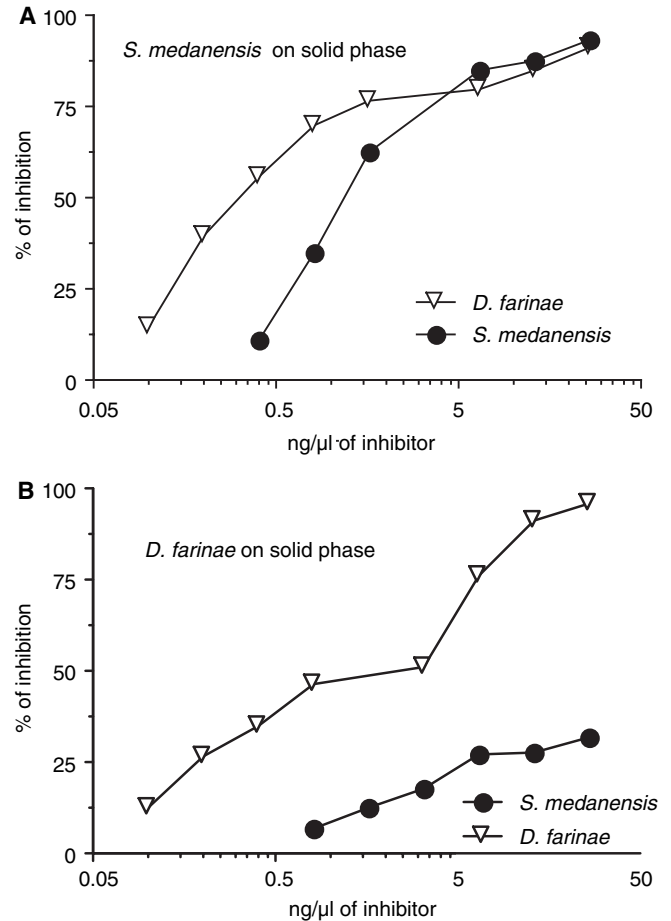


Figure 6. RAST inhibition curve with (A) *S. medanensis* and (B) *D. farinae* on solid phase (bound to the microtiter wells). The serum pool was adsorbed with different concentrations of inhibitor allergen: *D. farinae* or *S. medanensis* extracts.

allergic asthmatic patients living in a tropical environment. As *S. medanensis* mites occurs in low frequency in Cartagena the high prevalence of sensitization observed may be due to cross-reactivity with other mites, such as *B. tropicalis* and *Dermatophagoides* spp., the most prevalent mite species in this city.

The frequency of positive RAST to *B. tropicalis* in this study was similar to that reported in a previous study (3). It is also similar to that reported in other tropical city such as Cuba (19), and lower than the 92% reported in asthmatic children from Sao Paulo, Brazil (22). The levels of IgE to *B. tropicalis* were significantly higher to those for *S. medanensis*, which is consistent with the greater occurrence of *B. tropicalis* in Cartagena (13), and with the fact that in poly-sensitized patients, the IgE antibodies are mainly directed against proteins from the allergen source that act as main sensitizer.

The allergenic activity of *S. medanensis* extract was demonstrated by RAST in 71 of 97 sera; the results were also confirmed by immunoblot. In order to better analyze the number and frequency of allergenic components, we

selected 31 of these sera, because they showed a well-defined profile of IgE binding (Table 2). Our data indicate that whole body mite extract of *S. medianensis* has at least 14 allergens. The allergen with an apparent molecular weight of 30–31 kDa was detected by more than fifty percent of allergic patients (54.8%) and can be considered as a major allergen.

Several studies have reported a variable number of allergens for different domestic mite species using immunoblots; five allergens have been described for *A. siro* (23), 11 for *L. destructor* (24), 25 for *B. tropicalis* (5) and *Euroglyphus maynei* (25), and 32 for *D. pteronyssinus* (26). The fourteen allergens of *S. medianensis* detected in this study suggest that this mite has an important allergenic load and sensitizing potential, comparable with that of the more common mite species involved in allergic respiratory diseases. As was described for *B. tropicalis* extract (5), most of the sera detected between four and nine allergens in the *S. medianensis* extract, confirming the heterogeneity of the sensitization by domestic mite allergens. As the number of allergens identified in an allergenic extract depends on the population studied and the number of sera tested, the possibility exist that other allergenic components could be identified in *S. medianensis* by evaluating a larger number of sera and patients from other regions.

The RAST inhibition demonstrated that *B. tropicalis* inhibited 87.2% of IgE binding to *S. medianensis* extract, however only 32% of inhibition was achieved by *S. medianensis* when *B. tropicalis* was on solid phase. This can be explained by the fact that *B. tropicalis* is more prevalent than *S. medianensis* in Cartagena, influencing the sensitization process to each species. Indeed, *B. tropicalis* is an important sensitizer in the allergic populations of Carta-

gena (15), and high levels of its allergens have been found all the year around in house dust samples from this city (27).

Suidasia medianensis inhibited four allergens when *B. tropicalis* was used on the solid phase in immunoblot inhibition experiments. However, *B. tropicalis* inhibited 12 allergens when *S. medianensis* was used on the solid phase. Like RAST inhibition results, immunoblot inhibition data suggest that *B. tropicalis* is a stronger inhibitor of IgE binding to *S. medianensis* allergens than *S. medianensis* to *B. tropicalis*, implying that patients were mainly sensitized to *B. tropicalis*. RAST inhibition an immunoblot inhibition results also showed an important degree of cross-reactivity between *S. medianensis* and *D. farinae* involving six to nine allergens, being *D. farinae* the stronger inhibitor of IgE binding.

The three allergic patients who were sensitized to *S. medianensis* but not to *B. tropicalis* and the 13 who were sensitized to *B. tropicalis* but not to *S. medianensis*, were also sensitized to *D. farinae* and *D. pteronyssinus*. These results are consistent with previous finding regarding the important role of *B. tropicalis* and *Dermatophagoides* spp. as main sensitizer in our population (15, 21), to establish a species specific role to *S. medianensis* allergens further studies should be done. The allergenic potential of this mite species has been demonstrated in this study, therefore in regions where *S. medianensis* is endemic the clinical role of its allergens should be investigated.

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