

Original article

IgE cross-reactivity between *Ascaris* and domestic mite allergens: the role of tropomyosin and the nematode polyprotein ABA-1

Background: Analysis of cross-reactivity between the nematode *Ascaris* ssp. and dust mites, two important allergen sources in the tropics, will contribute in understanding their influence on asthma and atopy. The objective of this study was to investigate immunoglobulin E (IgE) cross-reactivity between *Ascaris* and two domestic mites in the tropics.

Methods: Sera from 24 asthmatic patients were used in ELISA and immunoblotting IgE-binding inhibition assays using *Ascaris*, *Blomia tropicalis* and *Dermatophagoides pteronyssinus* extracts and the recombinants Blo t 10, ABA-1 and Blo t 13 as competitors. Identification of *Ascaris* allergens was confirmed by mass spectrometry (LC-MS/MS).

Results: We detected at least 12 human IgE-binding components in *Ascaris* extract. *Blomia tropicalis* and *D. pteronyssinus* inhibited 83.3% and 79% of IgE-binding to *Ascaris*, while *Ascaris* inhibited 58.3% and 79.3% to *B. tropicalis* and *D. pteronyssinus* respectively. Mite tropomyosin inhibited 85% of IgE-binding to *Ascaris*. Affinity-purified human IgE to rBlo t 10 identified an allergen of 40 kDa in *Ascaris* extract, further confirmed as tropomyosin by LC-MS/MS. We found no evidence of IgE cross-reactivity between rABA-1 and any allergen component in mite extracts, including rBlo t 13.

Conclusions: There is cross-reactivity between *Ascaris* and mites, determined by several allergens including tropomyosin and glutathione-S-transferase. In addition to its potential impact on asthma pathogenesis, *Ascaris* infection and mite allergy diagnosis relying on the determination of specific IgE could be affected by this cross-reactivity. ABA-1 has no cross-reactive counterpart in mite extracts, suggesting its usefulness as a more specific marker of *Ascaris* infection.

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Allergy and asthma are phenotypes influenced by the environment. Mite sensitization is a risk factor for asthma, especially in tropical regions, where exposure to allergens from *Blomia tropicalis* and *Dermatophagoides pteronyssinus* is perennial (1–3). Some surveys have found that infection by *Ascaris lumbricoides* is a risk factor for asthma and atopy while others suggest the contrary (4). However, scarce information regarding cross-reactivity between allergens of mites and *Ascaris* is available, even though it could influence diagnosis of nematode infection and mite allergy and modify the natural history of mite-induced asthma. Analysis of this cross-reactivity will contribute to define, for example, the origin of the strong immunoglobulin E (IgE) responses to both sources in asthmatics (5, 6) and to find more specific reagents for diagnosis. Among potential cross-reactive proteins are tropomyosins,

paramyosins, glutathione transferases, proteases and fatty acid-binding proteins (FABPs). Cross-reactivity with mite tropomyosins can be evaluated because some of them have been cloned (7–10). For example, tropomyosin Blo t 10 is an important allergen from *B. tropicalis* (8) and has 73% amino acid sequence identity with the *A. lumbricoides* tropomyosin (11), therefore, it is likely to be cross-reactive with the homologous counterpart in the extract.

ABA-1 (Asc s 1), an allergen of *Ascaris* ssp., is a member of the nematode polyprotein allergen/antigens and has fatty acid-binding properties (12, 13). It has been used as serological marker of *Ascaris* infection (14–16). However, although it has only been found in nematodes, there are no studies of cross-reactivity with mite's FABPs (e.g. Blo t 13) or other allergens. The objective of this study was to evaluate the allergenic cross-reactivity

between mite and *Ascaris* extracts and to identify allergens of that cross-reactivity using whole extracts and recombinant allergens.

Methods

Sera

Sera of 24 asthmatic patients from Cartagena, a tropical city of Colombia were selected for preparing pools according to their IgE levels to *Ascaris*, *D. pteronyssinus*, *B. tropicalis*, rBlo t 10, rBlo t 13 and rABA-1 (Table 1). Asthma diagnosis was done as described previously (17). A pool of five sera from healthy nonallergic subjects was used as negative control. For correlation studies with rBlo t 10, 32 sera double positive to *Ascaris* and *B. tropicalis* were included. Subjects lived in an urban, nonindustrialized setting and belong to the lower three socioeconomic strata. The Bioethics Committee of the University of Cartagena approved the study and informed signed consent was obtained.

Ascaris and mite extracts

Ascaris extract was prepared by acetone–saccharose precipitation method (18). As it is the source currently employed for *in vitro* testing because of its close relationship with *A. lumbricoides*, *Ascaris suum* extract was used. Adult worms were washed in sterile saline with penicillin and streptomycin and homogenized in 4 ml of 0.25 M saccharose per gram of parasite. Extraction was performed by shaking the homogenate in cold acetone and leaving it for 20 min in cold bath (–80°C); then the sediment was resuspended in cold acetone, shaken and kept for 1 h in cold bath. The sediment was resuspended in acetone and centrifuged for 15 min at 9500 *g*, lyophilized and reconstituted in phosphate-buffered saline (PBS) pH 7.2 in a proportion of 0.4 ml per initial volume of homogenate. After shaking in cold for 16 h, it was centrifuged for 1 h at 9500 *g* (4°C). The supernatant was dialysed for 24 h against PBS pH 7.2 in a 3500 cutoff membrane (Spectrum Medical Industries, Los Angeles, CA, USA). Protein concentration (1.8 mg/ml) was determined by Lowry method. The lyophilized extract was kept at –20°C until used. *Dermatophagoides pteronyssinus* and *B. tropicalis* extracts were produced as described previously (19). To evaluate the effect of

Table 1. Immunological characteristics of sera

Serum pool A*							
Code	Age	Gender	IgE to <i>Blomia tropicalis</i> (kUA/l)	IgE to <i>Dermatophagoides pteronyssinus</i> (kUA/l)	IgE to <i>Ascaris</i> (kUA/l)	Total IgE (IU/ml)	
A842	48	M	26.3	11.4	16.8	494.6	
A1129	34	F	18.5	93.9	44.7	926.3	
A1072	48	F	>100	22.1	17.1	139.8	
A885	23	F	2.6	2.2	17.9	349.2	
A678	52	M	6.52	94.5	0.05	219.9	
A786	15	F	2.7	20.1	26.2	1259.4	
A303	37	F	9.0	1.3	3.1	196.3	
A658	42	F	2.0	1.82	8.9	718.0	
A765	10	M	61.6	42.1	19.0	3091.0	
Serum pool B and C†							
Code	Age	Gender	IgE to <i>B. tropicalis</i>	IgE to <i>D. pteronyssinus</i>	IgE to <i>Ascaris</i>	Total IgE (IU/ml)	IgE to Blo t 10 (OD)
A1014	43	F	37.4	54.7	42.8	714.8	3.61
A859	50	F	85.1	7.2	8.0	1013.9	3.64
A996	24	M	17.6	>100	0.34	898.1	0.55
A320†	17	F	20.8	9.8	8.0	2896.3	3.67
A599†	29	F	>100	>100	20.0	4182.5	0.59
A306†	22	M	21.6	23.9	10.3	7709.8	3.66
A1068	15	M	>100	98.2	30.9	1001.5	2.46
A697	47	F	27.4	26.1	1.75	1223.8	3.07
A504	35	M	16.7	11	3.78	254.6	0.21
A1015	57	M	0.31	16.3	0.91	8266.0	0.15
Serum pool D							
Code	Age	Gender	IgE to <i>B. tropicalis</i>	IgE to <i>D. pteronyssinus</i>	IgE to <i>Ascaris</i>	IgE to rBlo t 13 (OD)	IgE to rABA-1 (OD)
A896	28	F	16.6	>100	5.79	3.41	0.73
A996	24	M	17.6	>100	0.34	2.76	0.35
A543	15	M	15.6	10.6	7.67	1.40	0.61
A1015	57	M	0.31	16.3	0.91	0.11	0.43
A697	11	M	27.4	26.1	1.75	1.32	0.62

OD, optical density units.

*The pool A was prepared with these nine sera and pool B.

†Serum included in pool C.

IgE-binding cross-reactive carbohydrates, periodate oxidation of the extracts was performed under mild conditions (20, 21). Briefly, lyophilized extracts were diluted in sodium metaperiodate (Sigma, St. Louis, MO, USA) 0.05 M acetate buffer pH 4.5 to a final concentration of 10 mM, incubated for 1 h in the dark and then dialysed at 4°C.

Purified recombinant allergens: rABA-1, rBlo t 10 and rBlo t 13

cDNA encoding ABA-1 from *A. suum* was cloned into pGEX-1λT, and expressed as a glutathione-S-transferase (GST) fusion protein in *Escherichia coli* BL21 (Invitrogen Corporation, Carlsbad, CA, USA). It represents a single A-type repeat with few amino acid differences from the ABA-1 of *A. lumbricoides* and fully immunologically cross-reactive (22). Expression was induced with Isopropyl β-D-thiogalactopyranoside (0.1 mM), 4 h at 37°C. The rABA1 was purified with glutathione-sepharose beads and digested with thrombin; the nonfusion-protein was dialysed and kept at -20°C. rBlo t 10 was expressed in *E. coli* (8). rBlo t 13 was also expressed as fusion protein with GST as described previously (23).

Specific IgE to extracts and recombinant allergens

Total IgE was determined by duplicate using an ELISA kit (RIDASCREEN, R-Biopharm, Germany) according to the manufacturer instructions. Specific IgE to mite extracts (5 µg/well), *Ascaris* extract, rBlo t 13, rBlo t 10 and rABA1 (1 µg/well) were determined by indirect enzyme-linked immunosorbent assay (ELISA) as described previously (17). Immunoglobulin E antibodies to mites and *Ascaris* extracts were also determined by ImmunoCAP (Phadia AB, Uppsala, Sweden).

ELISA inhibition

Pools were adsorbed with increasing concentrations of inhibitors. After adsorption for 10 h at 4°C, 100 µl were loaded into wells coated with the relevant antigen and the plates incubated overnight. Antibodies bound to solid phase were measured by ELISA and the results were expressed in percentage of inhibition, calculated as follows: per cent of inhibition = $\frac{\text{OD without inhibitor} - \text{OD with inhibitor}}{\text{OD without inhibitor}} \times 100$. All assays included bovine serum albumin (BSA) as nonrelated inhibitor and GST as allergen control in experiments using Blo t 10/GST.

Immunoblotting inhibition

Proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (20 µg/lane) in a Mini-Protean 3 Electrophoretic Cell (Bio-Rad, Hercules, CA, USA). Samples were loaded with 5%-β-mercaptoethanol for reducing conditions. Proteins were electro-transferred onto 0.45 µm pore size nitrocellulose membranes using the Mini Trans-Blot Cell (Bio-Rad) with buffer 25 mM Tris-HCl, 192 mM glycine, 20% MeOH (pH 8.3). Membranes were blocked with 3% BSA/PBS, washed with 0.1% TPBS (Tween-PBS) and incubated overnight with serum pool. Strips were washed and incubated with alkaline-phosphatase-labelled anti-IgE (1 : 500) in buffer 0.5% TPBS, 0.2% BSA and then washed with buffer 0.15 M NaCl 0.05 M Tris-HCl (pH 7.5). Allergenic components were revealed using freshly prepared *p*-nitro-blue-tetrazolium and 5-bromo-4-chloro-3-indoyl-phosphate dissolved in alkaline-phosphatase buffer (pH 9.5) during 3 min. For immunoblotting inhibition, strips were incubated with 2 ml of each pool,

adsorbed (5 h) with 100 µg/ml of inhibitor. All assays included BSA as nonrelated control inhibitor and negative control serum. Estimation of molecular weight was done by calculating relative mobility according with migration of known MW standards (Broad Range Standards, Bio-Rad).

rBlo t 10 affinity-purified antibodies and immunoblotting

Affinity-purified IgE to rBlo t 10 was used to identify tropomyosin in *Ascaris* extract. Four microgram of allergen were electro-transferred to a nitrocellulose membrane and incubated overnight with a pool prepared with seven rBlo t 10 reactive sera, adsorbed with *Schistosoma japonicum*-purified GST. Antibodies bound to rBlo t 10 were eluted with 2 ml of 100 mM glycine (pH 2.5). After neutralization with 1 M Tris-HCl (pH 8.0), 4 mg/ml of BSA were added and the eluate was incubated with nitrocellulose strips containing *Ascaris* and mite extracts, rBlo t 10 and rBlo t 5.

LC-MS/MS peptide sequencing

Protein bands were excised and in-gel digested using the Proteo-Extract All-In-One Trypsin Digestion Kit (Calbiochem, San Diego, CA, USA). Tryptic peptides were separated by reversed phase-capillary high-performance liquid chromatography (HPLC, Nanoease Symmetry 300™ trap column and Nanoease Atlantis dC18™ separating column; Waters, Milford, MA, USA) directly coupled to an ElectroSpray ionization-quadrupole-time of flight mass spectrometer (Q-ToF Ultima Global; Waters). Data acquisition and instrument control was done with the MASSLYNX software V4.1 (Waters). The instrument was calibrated with the fragment ions of 22-Fibrinopeptide B (Sigma). Peptides were eluted with an acetonitrile gradient (Solvent A 0.1% v/v formic acid / 5% v/v acetonitrile, solvent B 0.1% v/v formic acid / 95% v/v acetonitrile; 5–45% B) and directly subjected to ionization and mass spectrometry. Data were acquired in the data-directed analysis mode in the mass range from 350 to 1900. Fragmentation time of the peptides was 4 s per ion, four ions were fragmented simultaneously. Peptide fragmentation data were acquired in the mass range from 50 to 1900. Survey and fragment spectra were analysed using the software PROTEINLYNX Global Server version 2.2.5 (Waters) with automatic and manual data validation. For sequence identification, Swiss-Prot and TrEMBL database were used.

Results

Human mite-allergic sera identify several IgE-binding components in *Ascaris* extract

Under reducing conditions, we detected 16 IgE-binding components in the *Ascaris* extract, those of 40, 34 and 23 kDa being the strongest (Fig. 1A). The same pool identified 19 components in *D. pteronyssinus* extract (116, 102, 95, 90, 81, 66, 60, 54–55, 50, 43, 41, 37, 35, 33, 29–30, 18, 17, 14–15 and 13 kDa) and 21 components (136, 102, 97, 84, 75, 50, 46, 45, 41, 39, 36, 31, 28, 25, 22, 21, 19, 17, 15, 14 and 11 kDa) in *B. tropicalis*. Under nonreducing conditions, the pattern of IgE-binding in the *Ascaris* extract was slightly different, being 12 (200, 116, 83, 77, 60, 58, 40, 34, 33, 23, 15 and 10 kDa) clearly visible (Fig. 1A). This was also observed with mite

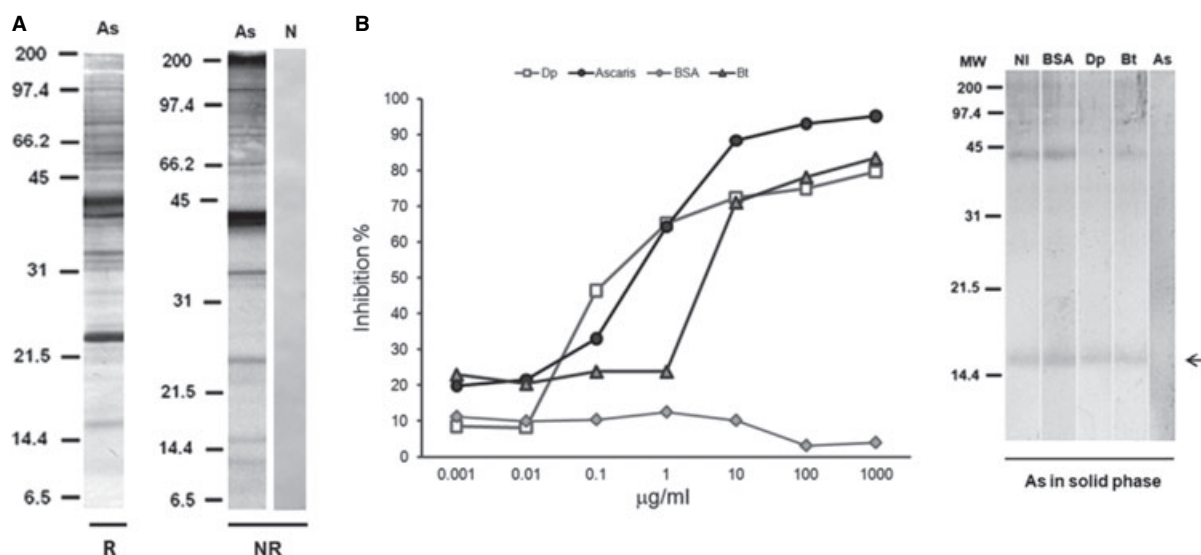


Figure 1. (A) Immunoblotting of *Ascaris* and mites using pool A (1 : 5). *Ascaris* extract under reducing (R) and nonreducing (NR) conditions. Under reducing conditions bands of 200, 175, 144, 130, 100, 97, 70, 61, 53, 47, 40, 34, 33, 31, 23 and 15 kDa are visible in *Ascaris* extract. As, *Ascaris*; Dp, *D. pteronyssinus*; Bt, *B. tropicalis*; N, negative control serum. (B) *Ascaris* in solid phase, pool B, dilution 1 : 20, the species-specific allergen of 15 kDa is pointed by the arrow. The names of inhibitors are shown above each lane, NI: no inhibited.

extracts (data not shown) consistent with previously reported data (24).

There is cross-reactivity between *Ascaris* and domestic mite allergens

Blomia tropicalis and *D. pteronyssinus* extracts inhibited 83.3% and 79% of IgE-binding to *Ascaris* (Fig. 1B), while *Ascaris* extract inhibited 58.3% and 79.3% of IgE-binding to *B. tropicalis* and *D. pteronyssinus*. We also analysed the inhibition of IgE-binding to *Ascaris* using pool C, obtaining 92.6% and 93% inhibition with *D. pteronyssinus* and *B. tropicalis*. Using pool B and periodate-treated extracts, *B. tropicalis* and *D. pteronyssinus* inhibited 68.4% and 50.6% of IgE-binding to *Ascaris* while *Ascaris* extract inhibited 63% of IgE-binding to mite extracts (data not shown).

There are several cross-reactive allergens in mite and *Ascaris* extracts

To investigate which allergens were cross-reactive we did immunoblotting inhibition assays. We observed the inhibition of a 40 kDa allergen and no inhibition of a 15 kDa allergen (Fig. 1C). Using pool C, there was total inhibition of IgE-binding to the 40 kDa allergen and other allergenic components (200, 97 and 23 kDa) of *Ascaris* by both mites (data not shown). To increase the IgE repertoire, we performed experiments using pool A. Under reducing conditions, mite extracts inhibited IgE-binding to allergens of 200, 175, 144, 100, 97, 61, 53, 40, 33 and 31 kDa (Fig. 2A). Under nonreducing conditions,

mites inhibited IgE-binding to components of 200, 116, 77, 58 (only *D. pteronyssinus*), 40, 33 and 23 kDa, while components of 10, 13, 15, 34 and 60 kDa were not inhibited (Fig. 2B). The 200, 40, 33 and 23 kDa allergens were consistently inhibited by both mites in reducing and nonreducing conditions. The *Ascaris* extract partially inhibited the IgE-binding to 11 allergens of *D. pteronyssinus* (Fig. 2C) and to another 11 of *B. tropicalis* with total inhibition to those of 22 and 19 kDa (Fig. 2D). No inhibition of IgE-binding to allergens of 15 kDa or those ranging from 14 to 17 kDa was found.

Tropomyosin is a cross-reactive allergen between *Ascaris* and domestic mites

A significant correlation between IgE levels to rBlo t 10 and *Ascaris* was found (Spearman = 0.61, $P = 0.0001$). rBlo t 10 in solid phase showed 95% homologous inhibition, 71% with *B. tropicalis*, 72% with *D. pteronyssinus* and 37% with *Ascaris* (Fig. 3A). With *Ascaris* in solid phase, 91% homologous and 75.2% inhibition with rBlo t 10/GST was found (Fig. 3B). Most of the IgE reactivity was directed to the 40 kDa allergen in *Ascaris* extract, which was strongly inhibited by mites. Therefore, we performed immunoblotting inhibition by preadsorption with rBlo t 10-GST. The IgE-binding to the 40 kDa allergen was completely inhibited by Blo t 10-GST while the 34 kDa allergen remained unchanged (Fig. 3B). Additionally, using human-specific IgE to rBlo t 10 the 40 kDa allergen was detected in the extract, as well as the rBlo t 10-GST fusion protein while no signal was obtained with the nonrelated allergen rBlo t 5 (Fig. 3C). By tandem

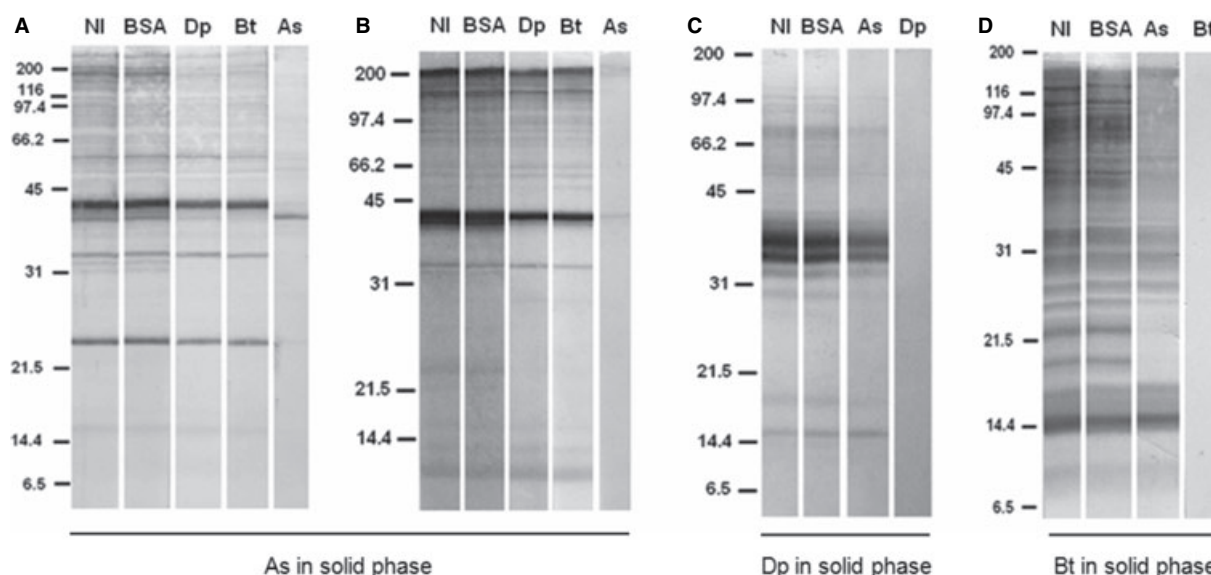


Figure 2. Immunoblotting inhibition using pool A (1 : 10), under (A) reducing and (B) nonreducing conditions. (C) Pool 1 : 50. *Ascaris* extract inhibited IgE-binding to allergens 116, 95, 90, 81, 66, 54–55, 37, 35, 33, 29 and 18 kDa, (D) Pool 1 : 20. *Ascaris* inhibits IgE-binding to 116, 102, 84, 77–75, 71, 43, 41, 33–34, 24, 22 and 19 kDa allergens.

LC-MS/MS and subsequent database search, this allergen was confirmed as *A. lumbricoides* tropomyosin (TrEMBL accession number A8DKY0) with a match of 10 peptides and sequence coverage of 30.9% (Fig. 4).

Anti-tropomyosin antibody also recognized a 23 kDa allergen in the *Ascaris* extract, corresponding to GST, indicating that the adsorption of the pool was incomplete. This band was also recognized by pool C and inhibited by mite extract. By tandem LC-MS/MS, eight peptides of the 23 kDa allergen matched to GST from *A. suum* (P46436), resulting in a sequence coverage of 30.1%.

ABA-1 is not cross-reactive with mite allergens

We consistently found no inhibition of allergenic components of 15, 13 and 10 kDa. These *Ascaris*-specific allergens were all identified by tandem LC-MS/MS as fragments of ABA-1 (Fig. 4). To investigate whether this FABP is cross-reactive with any component in mite extracts, we analysed the IgE-binding inhibition by preadsorption with *B. tropicalis*, *D. pteronyssinus* and rBlo t 13. We observed total inhibition of IgE-binding when *Ascaris* and the homologous molecule were used as competitors, but, there was no inhibition with *B. tropicalis*, *D. pteronyssinus* or Blo t 13, suggesting that mite extracts do not contain ABA-1 epitopes and this allergen is not cross-reactive with the mite FABP, Blo t 13 (Fig. 5A). Immunoblotting inhibition with rBlo t 13 in solid phase showed total homologous inhibition and partial inhibition with its native equivalent in the *B. tropicalis* extract, but no inhibition with *Ascaris* extract or rABA-1 (Fig. 5B,C).

Discussion

We analysed the allergen cross-reactivity between the two most important allergenic mites in the tropics and the nematode *Ascaris*, using sera from mite-allergic patients living in a place where exposure to mites is perennial and naturally infection with this parasite is endemic. As a result, we found that (i) *Ascaris* extract has at least 12 human IgE-binding components; (ii) there is important cross-reactivity between the mites *B. tropicalis* and *D. pteronyssinus* and *Ascaris*; (iii) there are at least four allergens of *Ascaris* involved in this cross-reactivity, among them tropomyosin and GST and (iv) the allergen ABA-1 from *Ascaris* is not cross-reactive with components of mite extracts.

In *Ascaris* extract, we detected 16 IgE-binding components under reducing and 12 under nonreducing conditions. Thus, it is likely that the actual number of individual allergens is that found under nonreducing conditions, when allergens are less denatured. This number and recognition pattern is similar in *Anisakis simplex* under nonreducing conditions (25). However, it is necessary to analyse the frequency of sensitization at the population level to obtain information about the patterns of sensitization in patients and controls.

We found that cross-reactivity with *Ascaris* is high for both mites but it is important to consider whether this is related to cross-reactive carbohydrates. Our assays using periodate-oxidized extracts showed a low reduction in the level of cross-reactivity compared with the crude extracts, suggesting that carbohydrate groups are involved, but most of the cross-reactivity is

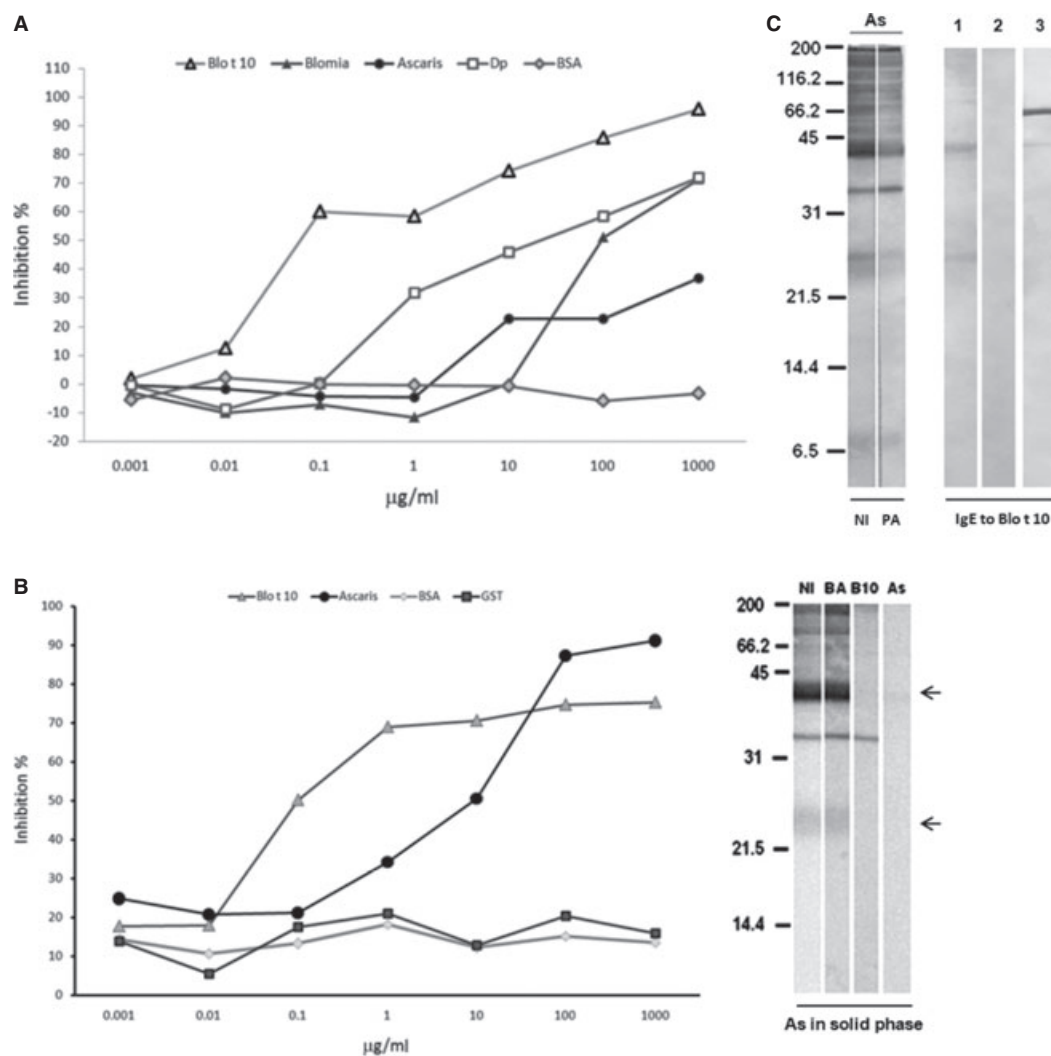


Figure 3. (A) ELISA inhibition using pool C and rBlo t 10 in solid phase. (B) *Ascaris* in solid phase. (C) Anti-tropomyosin IgE-binding components in *Ascaris* (lane 1); no related allergen rBlo t 5 (lane 2) and rBlo t 10/GST (lane 3). Preabsorption (PA) of pooled sera with rBlo t 10/GST decreases the intensity of IgE recognition of the 40 kDa allergen of *Ascaris* extract.

because of protein epitopes, which is supported by our experiments using recombinant nonglycosylated allergens.

To identify the cross-reactive allergens, we did immunoblotting inhibitions using the same sera of ELISA experiments and mass spectrometry analysis. Under reducing conditions, mite extracts inhibited the same 10 allergens of *Ascaris* and the same seven allergens under nonreducing conditions. Four of them (200, 40, 33 and 23 kDa) were inhibited by mites in both conditions, suggesting that they are distinct molecules. Thus *Ascaris* extract has several mite-cross-reactive allergens, among them the 40 kDa allergen, which exhibited the highest intensity of IgE-binding. We demonstrated, for the first time, cross-reactivity between rBlo t 10 and this 40 kDa homologous allergen in the *Ascaris* extract, confirmed as *Ascaris* tropomyosin by LC-MS/MS. Our results are in

agreement with the reported 40.9 kDa native tropomyosin of *A. simplex* (26) and to that described for *A. suum* (27). In mite extracts, we also observed allergens with the molecular mass of tropomyosins (7, 8), partially inhibited by *Ascaris* extract, as was described in cross-reactivity experiments between *A. simplex* and four mite species (25).

Cross-reactivity between mites and nematodes has been suspected for a long time and has only been described for *D. pteronyssinus* and *A. simplex* (25). However, cross-reactivity between mites and *Ascaris* had not been demonstrated, even though several studies have suggested a relationship between specific IgE to this nematode and atopy, allergic sensitization, wheezing, asthma and asthma severity (4, 28). In this regard, our findings have two important implications that deserve further analysis.

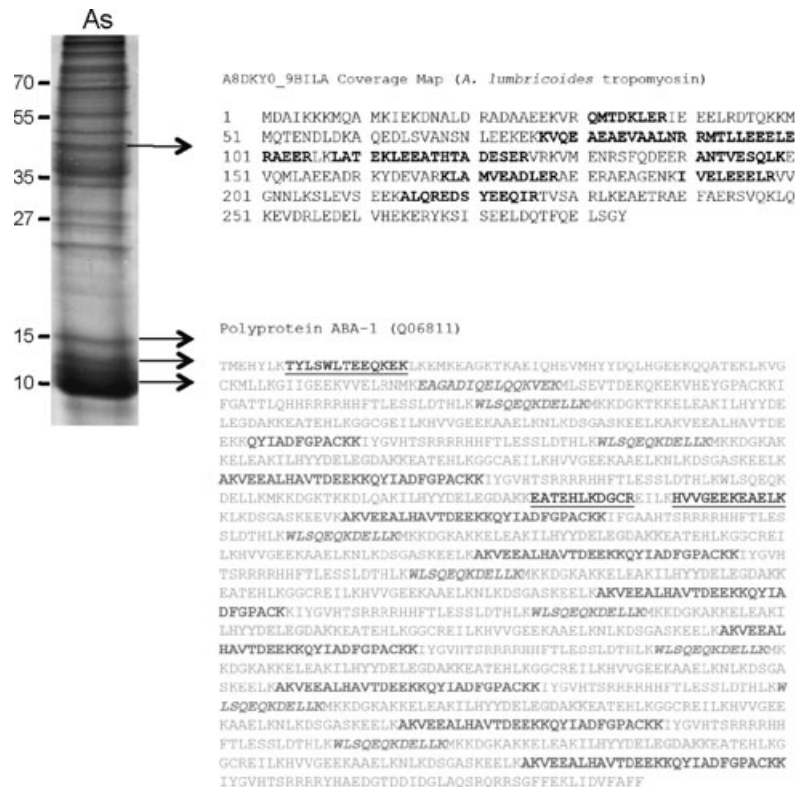


Figure 4. Coomassie staining of *Ascaris suum* extract showing the bands selected for in-gel digestion and analysis by LC/MS/MS. Coverage map obtained by the resulting sequenced peptides are shown for the tropomyosin (40 kDa) and ABA-1 (15, 13 and 10 kDa).

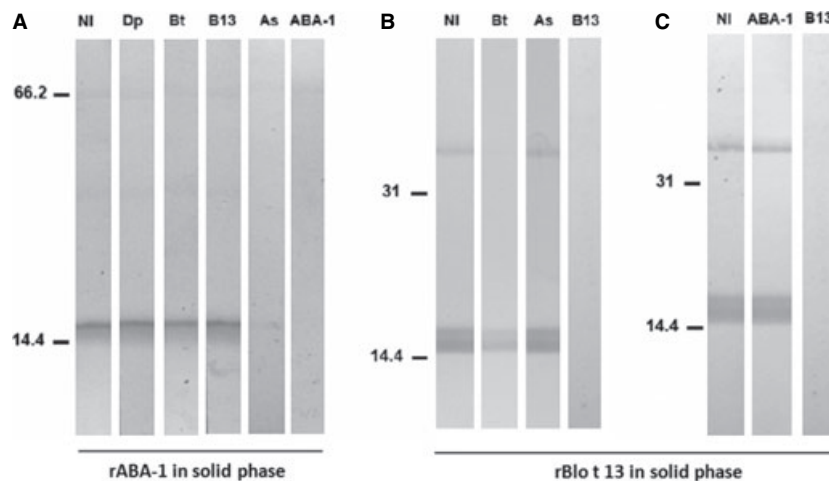


Figure 5. Immunoblotting inhibition using whole extracts and purified *Ascaris* and mite FABPs. (A) ABA-1 in solid phase (1 µg/lane), (B) and (C) Blo t 13 in solid phase (1 µg/lane). The names of inhibitors are above each lane. B13, Blo t 13. All the experiments were done with pool D, double positive to ABA-1 and Blo t 13.

First, in surveys using IgE to *Ascaris* extract as a marker of infection, the results could be biased for cross-reactive allergens. The interpretation of serological tests for this infection using the nematode extract currently considers cross-reactivity with other nematodes (29, 30) but should also take into account cross-reactivity with

mites. We found no components sharing IgE-epitopes with ABA-1 in mite extracts, suggesting that this molecule is more specific for evaluating the infection with *Ascaris*. Secondly, in a tropical environment, where parasites are common and people are co-exposed to mites, the cross-reacting allergens could provide a

permanent boosting, promoting synthesis of high specific IgE levels and influencing the inception of allergy and the clinical evolution of asthma.

Most reports on cross-reactivity between mites and other invertebrates suggest that mites are the primary sensitizer (31). It is difficult to define this point using whole extracts and pooled sera, but our results support this idea. In most sera, IgE levels to mites are higher than to *Ascaris* (Table 1) and inhibitions were stronger when using mite extracts than *Ascaris* extract. However, prospective investigations are necessary to identify the source inducing the primary response.

In summary, we found cross-reactivity between *Ascaris* and domestic mites, contributing with data about aller-

gens potentially involved in asthma and suggesting a more specific reagent for serological diagnosis of *Ascaris* infection.

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