

Parasite Infections, Allergy and Asthma: A Role for Tropomyosin in Promoting Type 2 Immune Responses

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Keywords

IgE cross-reactivity · Tropomyosin · Cockroach · Asthma · Recombinant allergens · *Ascaris lumbricoides*

Abstract

Introduction: The relationship of parasite infections and promotion or protection from allergy and asthma is controversial. Currently, over 1.5 billion people are infected with parasites worldwide, and *Ascaris lumbricoides* is the most frequent soil-transmitted helminth. **Objectives:** To evaluate the biological activity of recombinant *A. lumbricoides* tropomyosin and investigate IgE cross-reactive responses to tropomyosins by means of microarray methodology for the detection of sensitization to allergen components. **Methods:** Forty patients 12–75 years of age (25 males) with asthma and/or rhinitis and 10 nonallergic control subjects participated in this study. All patients presented positive skin tests to cockroach extracts and underwent skin prick testing (SPT) with recombinant (r) tropomyosins rPer a 7 from *Peri-*

planeta americana and rAsc I 3 from *A. lumbricoides*, at 10 µg/mL. IgE to cockroach and parasite tropomyosins were measured by chimeric ELISA and ImmunoCAP-ISAC, and total IgE was quantitated by ImmunoCAP. Agreement of results was assessed by κ statistics. **Results:** Recombinant *A. lumbricoides* showed biological activity, inducing positive skin tests in 50% patients with asthma and/or rhinitis. IgE to cockroach and parasite tropomyosins were detected in 55–62% of patients. There was good-to-excellent agreement of results of SPT and IgE measurements by ELISA and ImmunoCAP-ISAC, with κ indices of 0.66–0.95. No skin test reactivity or IgE antibodies to tropomyosins were found in nonallergic individuals. **Conclusions:** Our results suggest that IgE responses to tropomyosin from *A. lumbricoides* may enhance reactivity to homologous allergens upon exposure by inhalation or ingestion, promoting allergic reactions and asthma, or increasing the severity of these clinical conditions.

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Introduction

Infections from intestinal parasites are still a major public health problem worldwide, leading to malnutrition, anemia, cognitive impairment, and a decreased response to vaccination [1]. It is estimated that 1.5 billion people in the world are currently chronically infected with at least 1 soil-transmitted helminth parasite [2], with young children from less-privileged communities being the most vulnerable. The relationship of infections from parasites, particularly those caused by helminths, and promotion or protection from allergy and asthma has been extensively debated over time [3–5]. Both conditions are usually associated with a strong type 2 immune response, characterized by IgE class-switching in B cells, and the production of type 2 cytokines including interleukin (IL)-4, IL-5, and IL13 by Th2 and ILC2 cells, and eosinophilia. Peculiarly, parasite infections also induce a regulatory response, with increases in regulatory T and B cells (Tregs and Bregs) as well as increased production of IL-10 and TGF- β , thought to aid parasite survival in the host [1].

Studies addressing the effects of parasite infections in allergy and asthma have led to conflicting results, and it is thought that the variability in outcomes may be due to several factors including the timing and duration of infection, the parasite load, host genetics, and helminth species [1–5]. *Schistosoma* infection, particularly, has been consistently associated with protection, as demonstrated by a decrease in allergy skin test positivity, a milder course of asthma, and lower rates of hospitalization due to asthma in endemic zones; these effects have been attributed to a regulatory response associated with a marked increase in IL-10 [1, 6].

On the other hand, such outcomes have not been observed as a result of *Ascaris lumbricoides* infection. Reduction in IL-10 production, increased prevalence of wheezing and asthma, bronchial hyperresponsiveness in children, and improvement of asthma symptoms after treating *Ascaris* infection have been reported [4, 7], but the attenuation of allergy skin test reactivity has also been described [1]. In addition, IgE sensitization to *Ascaris* and to cross-reactive tropomyosins has been associated with clinical indicators of asthma severity, and infection with *Ascaris* was found to increase IgE responses to mite-specific allergens in patients with asthma in Colombia [7–9].

Tropomyosins are panallergens present in invertebrates including shrimp, other crustacean and mollusks, mites, cockroaches and other insects, and parasites ([10, 11]. Our group identified *A. lumbricoides* tropomyosin as a major IgE-binding protein, particularly among children living in underprivileged conditions in northeast Brazil

with current parasite infections, but also among cockroach-allergic patients with asthma and/or rhinitis living in a nonendemic zone in southeast Brazil who presented without parasite infections on stool exams; IgE reactivity was 78.1 and 42%, respectively, in these 2 groups of patients [10]. We showed a strong correlation of IgE to recombinant tropomyosins from cockroach and *Ascaris* using a chimeric ELISA. Moreover, we detected high-level expression of tropomyosin in *A. lumbricoides* L3-larvae, the stage of pulmonary passage during *Ascaris* infection, using a monoclonal antibody directed to mite *Dermaphagoides pteronyssinus* tropomyosin [10]. The high degree of sequence identity of approximately 70% of *Ascaris* tropomyosin to cockroach and mite tropomyosins prompted us to hypothesize that there is IgE cross-reactivity, which could be clinically relevant in terms of influencing IgE responses in allergic diseases and asthma.

In this study, we aimed to evaluate the biological activity of recombinant *A. lumbricoides* tropomyosin and to expand our investigation of IgE cross-reactive responses to tropomyosins assessed previously by an in-house ELISA to a more widely used microarray methodology for the detection of sensitization to allergen components.

Subjects and Methods

Subjects and Skin Testing

A total of 40 patients, 12–75 years of age (15 females, 37.5%), with asthma and/or rhinitis participated in this study. Patients were selected from among those attending the Allergy Clinic at the Clinical Hospital of Ribeirão Preto Medical School in Brazil. All patients presented with positive skin tests to cockroach extracts of *Periplaneta americana* and *Blattella germanica* (1:20 w:v), and also to mite extracts of *D. pteronyssinus* (38/40) and *D. farinae* (39/40) (10,000 AU/mL) (Greer Laboratories, Lenoir, NC, USA). Patients underwent skin prick testing (SPT) with the recombinant (r) tropomyosin allergens rPer a 7 from *P. americana* and rAsc l 3 from *A. lumbricoides*, at a concentration of 10 μ g/mL, as established in a previous study [12]. Ten nonallergic, nonasthmatic subjects, 24–54 years of age (9 females, 90%), who presented with negative skin tests to a panel of inhalant allergens, including cockroach, were selected as negative controls. SPT was carried out on the forearm by using a prick lancetter (Allergopricks Inox, Flexor, SP, Brazil). A mean wheal diameter of ≥ 3 mm, accompanied by erythema, that developed within 15 min after the application of the allergen, was considered a positive reaction.

Recombinant Proteins

Recombinant *P. americana* and *A. lumbricoides* tropomyosins were expressed in *Pichia pastoris* using the pPIC9 vector, as previously described [10]. Briefly, *P. pastoris* cultures were grown at 30 °C with shaking, and supernatants were collected at 72 h. *Pichia*-expressed tropomyosins from *A. lumbricoides* and *P. americana* were purified from culture supernatants over separate mono-

Table 1. Demographic data, skin test results, and IgE antibodies to cockroach and *Ascaris/Anisakis* tropomyosins among cockroach-allergic patients with asthma and/or rhinitis

	Patients (n = 40)	Controls (n = 10)
Age, years	30 (12–75)	34 (24–54)
Female sex	15 (37.5)	9 (90) ^a
Diagnosis		
Asthma with rhinitis	29 (72.5)	0
Asthma	3 (7.5)	0
Rhinitis	8 (20)	0
Total IgE, IU/mL	838.4 (48–3,738)	28.6 (4.1–103)
Positive skin test to <i>D. pteronyssinus</i> extract ^b	38 (95)	0
Positive skin test to <i>D. farinae</i> extract	39 (97.5)	0
Positive IgE to <i>A. lumbricoides</i> by ImmunoCAP ^c	30 (74)	0
IgE to <i>A. lumbricoides</i> by ImmunoCAP, kU/L	11.5 (0.46–49)	undetectable
Positive skin test to rPer a 7	23 (57)	0
Positive skin test to rAsc l 3	20 (50)	0
Wheal diameter on skin test to rPer a 7, mm	6 (3–13)	negative
Wheal diameter on skin test to rAsc l 3, mm	5 (3–12)	negative
Positive IgE to rPer a 7 on ELISA	25 (62)	0
Positive IgE to rAsc l 3 on ELISA	25 (62)	0
IgE to rPer a 7 on ELISA, IU/mL	17 (0.92–183.3)	undetectable
IgE to rAsc l 3 on ELISA, IU/mL	10 (0.58–500)	undetectable
Positive IgE to Bla g 7 by ImmunoCAP-ISAC	22 (55)	0
Positive IgE to Ani s 3 by ImmunoCAP-ISAC	23 (58)	0
IgE to Bla g 7 by ImmunoCAP-ISAC, ISU-E	9.2 (1.1–69)	undetectable
IgE to Ani s 3 by ImmunoCAP-ISAC, ISU-E	5.2 (0.4–69)	undetectable

Values express n (%) or mean (range). All patients presented negative stool exams for parasites and denied any clinical history of previous parasitic infections. ^a There were significantly more female individuals in the control group ($p < 0.01$, Fisher's exact test). ^b Only 1 patient was double-negative to *D. pteronyssinus* and *D. farinae* on skin testing. This patient had undetectable IgE to *A. lumbricoides*. ^c 8/10 patients with negative *A. lumbricoides* IgE on ImmunoCAP were negative on skin tests to rAsc l 3, and 2/10 presented weak skin test reactions (i.e., a wheal diameter of 3 mm).

clonal antibody (mAb) affinity columns, using anti-tropomyosin mAb 1A6, which was originally raised against *D. pteronyssinus* tropomyosin, but recognizes tropomyosins from cockroach, mites, and shrimp [10]. Proteins were eluted with 0.005 mol/L glycine (pH 2.8) and analyzed by silver-stained SDS-PAGE using 8–25% gradient gels (Pharmacia PhastSystem).

Total IgE and Specific IgE to Recombinant Tropomyosins

Levels of total IgE and IgE to *P. americana* and *A. lumbricoides* were determined using the ImmunoCAP system (Thermo Fisher, Brazil), and results were expressed as IU/mL or kU/L. IgE antibodies to rPer a 7 and rAsc l 3 were measured by a chimeric ELISA, as previously described [10, 12]. In addition, IgE antibodies to allergen components were assessed by ImmunoCAP-ISAC (Thermo Fisher), and results were expressed as ISU (ISAC standardized units), according to the manufacturer's instructions. The original version of the test, comprising 103 natural or recombinant purified allergens, derived from 43 sources, was used in this study. In this fluoroenzymatic microarray system, allergens nBla g 7 from *B. germanica* (96.5% amino acid sequence identity to *P. americana* al-

lergen Per a 7) [13] and Ani s 3 from *Anisakis simplex* (98% sequence identity to *A. lumbricoides* antigen Asc l 3) [10] were available. Due to the high degree of sequence identity between Bla g 7 and Per a 7, and Ani s 3 and Asc l 3, IgE responses to these allergens were considered interchangeable, respectively.

Statistical Analysis

Agreement of results of skin tests and measurements of IgE to rPer a 7/nBla g 7 and rAsc l 3/rAni s 3 by ELISA and ImmunoCAP-ISAC was assessed by κ statistics; the κ index and its 95% confidence interval (CI) were calculated as previously described [12].

Results

In this study, a total of 40 patients underwent SPT with recombinant tropomyosins from *P. americana* (rPer a 7), and *A. lumbricoides* (rAsc l 3). Characteristics of patients

Table 2. Comparison of total IgE and specific IgE responses in patients with positive and negative skin tests to recombinant *A. lumbricoides* tropomyosin (rAsc l 3)

Parameter	Patients with positive skin tests to Asc l 3 (n = 20)	Patients with negative skin tests to Asc l 3 (n = 20)	p value
Total IgE, UI/mL	925.5 (67–3,738)	791.2 (48–2,610)	0.66
Wheal diameter on skin test for <i>D. pteronyssinus</i> extract, mm	7.9 (0–13)	7.5 (0–15)	0.5
Wheal diameter on skin test for <i>D. farinae</i> extract, mm	7.7 (3–13)	7.2 (0–13)	0.61
IgE to cockroach <i>P. americana</i> , kU/L	10.8 (0.7–44.3)	3.4 (0.38–32.1)	0.02*

Values express mean (range). All patients tested positive to *P. americana* extract, 38/40 tested positive to *D. pteronyssinus* extract, 39/40 tested positive to *D. farinae* extract (one double-negative), and 31/40 showed IgE to *P. americana* by ImmunoCAP. * Wilcoxon nonparametric test.

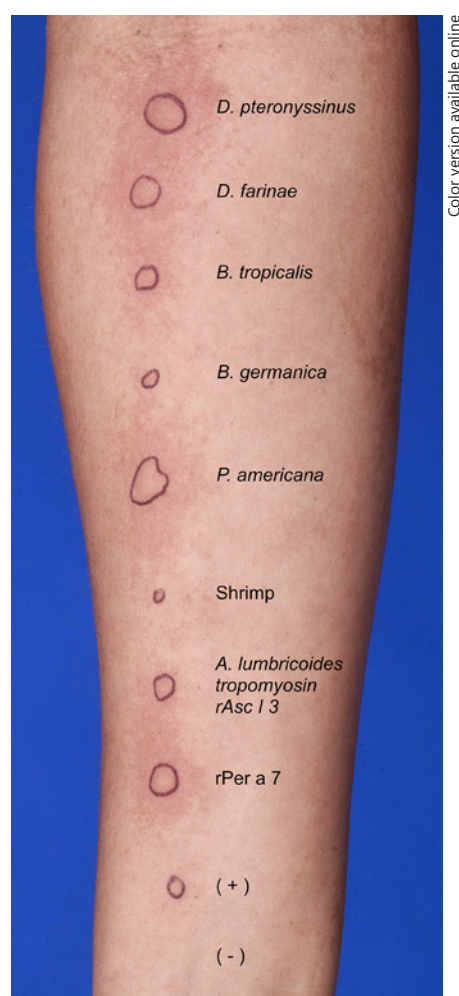


Fig. 1. Skin prick testing in a representative patient who participated in the study. Positive tests were observed for the recombinant tropomyosins from cockroach, rPer a 7, and from *A. lumbricoides*, Asc l 3.

and controls participating in the study are shown in Table 1. The mean level of total IgE was 838.4 and 28.6 IU/mL in patients and controls, respectively. There was a significant difference in female-to-male ratio between the groups, with more females in the control group (90%) than in the patient group (37.5%) ($p < 0.01$, Fisher exact test).

Of 40 patients tested, 23 (57%) and 20 (50%) showed positive on SPT to rPer a 7 and rAsc l 3, respectively. Comparing sensitization patterns in patients with positive and negative skin tests to *A. lumbricoides* tropomyosin, total IgE levels and mean wheal diameters on skin tests to *D. pteronyssinus* and *D. farinae* were not significantly different between the 2 groups, but IgE to *P. americana* was significantly higher in the group with positive skin tests than in those with negative skin tests to rAsc l 3 (Table 2). Ten nonallergic control subjects showed no skin reactivity to recombinant cockroach and *Ascaris* tropomyosins. Figure 1 shows the results of SPT in a representative patient participating in the study, who presented positive tests to rPer a 7 and rAsc l 3.

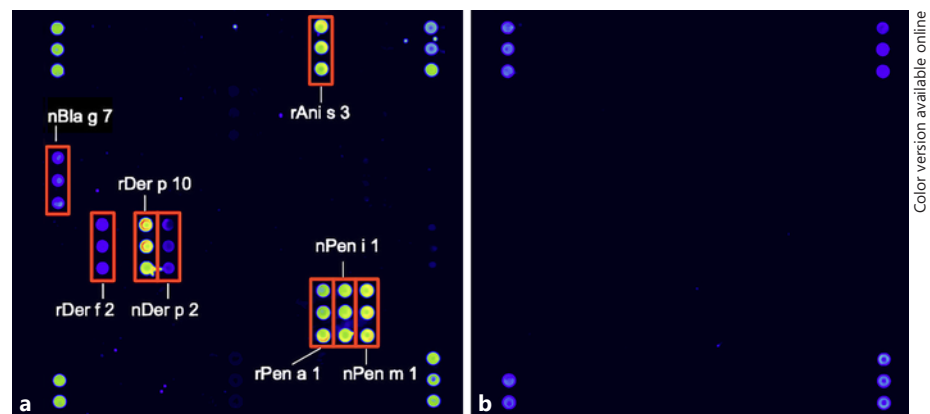
By chimeric ELISA, 25/40 patients (62%) had detectable IgE to rPer a 7 and rAsc l 3, respectively. Likewise, 22 (55%) and 23 (58%) of 40 patients had detectable IgE to rPer a 7 and rAsc l 3, respectively, on ImmunoCAP-ISAC (Table 1). Figure 2 shows the ImmunoCAP-ISAC profile of a representative patient who participated in the study. This patient presented high levels of IgE to tropomyosins from cockroach (rBla g 7) and *Anisakis* (Ani s 3), but also to mite (Der p 10) and shrimp (rPen a 1, nPen i 1 and nPen m 1) tropomyosins, within the range of 14–51 ISU, consistent with the moderate/high or very high levels proposed in the method (Fig. 2). In addition, she was also positive to the mite allergens Der p 2 and Der f 2. There was an excellent agreement of results of skin tests with

Table 3. Agreement of results of skin prick tests and measurements of IgE to cockroach and parasite tropomyosins by ELISA and ImmunoCAP-ISAC among patients with asthma and/or rhinitis allergic to cockroach

	Skin test to cockroach tropomyosin (rPer a 7)	Skin test to <i>Ascaris</i> tropomyosin (rAsc l 3)	IgE to rPer a 7 ELISA	IgE to rAsc l 3 ELISA	IgE to Bla g 7 ImmunoCAP-ISAC	IgE to Ani s 3 ImmunoCAP-ISAC
Skin test to cockroach tropomyosin (rPer a 7)	1.00	–	–	–	–	–
Skin test to <i>Ascaris</i> tropomyosin (rAsc l 3)	0.83 (0.66–1.00)	1.00	–	–	–	–
IgE to rPer a 7 ELISA	0.89 (0.76–1.00)	–	1.00	–	–	–
IgE to rAsc l 3 ELISA	–	0.75 (0.55–0.95)	0.66 (0.42–0.91)	1.00	–	–
IgE to Bla g 7 ImmunoCAP-ISAC	0.95 (0.84–1.00)	–	0.83 (0.66–1.00)	–	1.00	–
IgE to Ani s 3 ImmunoCAP-ISAC	–	0.84 (0.7–1.00)	–	0.66 (0.42–0.91)	0.95 (0.84–1.00)	1.00

Values express κ indices with 95% confidence intervals.

Fig. 2. ImmunoCAP-ISAC IgE reactivity profile of a representative patient and a control subject. **a** Representative result of ImmunoCAP-ISAC analysis of 1 of our patients allergic to cockroach and mite, who showed IgE reactivity to tropomyosins from cockroach, parasite, mite, and shrimp, and also to mite group 2 allergens. IgE results were: cockroach nBla g 7 tropomyosin 14 ISU; *Anisakis* rAni s 3 tropomyosin 25 ISU; mite rDer p 10 tropomyosin 51 ISU; shrimp tropomyosins rPen a 1, 23 ISU; nPen i 1, 28 ISU; nPen m 1, 36 ISU. Positive results were also found for rDer f 2, 10 ISU and nDer p 2, 6 ISU. **b** Results from 1 negative nonallergic, nonasthmatic control subject showing no reactivity to the 103 allergens tested on the microchip. The ImmunoCAP-ISAC test was performed with triplicates of each allergen, and the peripheral dots are internal controls.



cockroach and *Ascaris* recombinant tropomyosins, with a κ index of 0.83 (95% CI 0.66–1.00) (Table 2). Overall, the results of skin test and specific IgE measurements by both ELISA and ImmunoCAP-ISAC methods also showed good-to-excellent agreement, as judged by κ indices varying from 0.66 (95% CI 0.42–0.91) to 0.95 (95% CI 0.84–1.00) (Table 3).

Discussion

We demonstrated that recombinant *A. lumbricoides* tropomyosin, produced in *P. pastoris* presents biological activity, inducing positive SPT results in 50% of adult patients with asthma and/or rhinitis and allergic to cockroach. In addition, component analysis by ImmunoCAP-

ISAC revealed that 58% of these patients presented IgE to Ani s 3 (98% sequence identity to Asc l 3 from *A. lumbricoides*), with an excellent agreement of the results (κ index 0.84) of both methods assessing the IgE response. These results expand our previous observations that 42% of a group of patients with asthma, wheezing, and/or rhinitis, aged 2–52 years, presented IgE antibodies in sera to recombinant *A. lumbricoides* tropomyosin, using a chimeric ELISA [10].

The high degree of amino acid sequence identity and structural similarity among invertebrate tropomyosins could account for the cross-reactive IgE antibody responses, as suggested by our group and others [7, 10]. This IgE cross-reactivity among environmental or food allergens and helminth antigens may hamper the diagnostic value of measuring IgE responses to whole-aller-

gen extracts in populations from helminth-endemic areas [2]. However, it is possible that IgE cross-reactivity with tropomyosin from parasites, particularly *A. lumbricoides*, could account for the high frequency of sensitization to tropomyosins in Brazil, Colombia, and countries in Africa [7, 10, 12, 14], as opposed to other areas of the world including the USA and Taiwan where tropomyosins from mites and cockroach are minor allergens reacting to IgE in only 12–30% of patients.

One question that remains is whether IgE cross-reactive responses could have clinical significance and underlie the development of symptoms in sensitized individuals. We recently reported 3 patients with rhinitis and controlled asthma who denied ever having eaten shrimp, other crustacea, or mollusks their entire life, but presented strongly positive on SPT for shrimp. They also had positive SPT results and high IgE levels to mites and cockroach, and detectable IgE to shrimp tropomyosin [15]. Upon oral challenges with shrimp, 2 of the patients had a mild reaction, but the 3rd experienced severe anaphylaxis upon ingestion of a 20-mg cumulative dose of shrimp during an open challenge, requiring intubation and treatment in ICU. Allergic reactions upon ingestion of shrimp in the cases we reported (i.e., the asthma patients who had never eaten shrimp) suggest that IgE sensitization to mites and cockroach, primarily through the respiratory tract, and perhaps via parasite infection, could induce cross-sensitization to shrimp such that ingestion of shrimp could lead to a life-threatening systemic allergic reaction [15]. In keeping with our results, Tuano and Davis [16] described 4 mite-allergic patients with rhinitis, with and without asthma, who developed oral allergy syndrome upon the ingestion of shrimp, although their reaction did not progress to anaphylaxis during oral food challenge [16]. In this study, patients who presented positive skin test to *Ascaris* tropomyosin had significantly higher levels of IgE to cockroach *P. americana* than patients with negative skin test to this allergen, providing further support for the hypothesis of cross-sensitization.

One limitation of our study is the significantly higher frequency of females in our control group. However, we do not feel that this finding had an impact on our results. In adult patients, it is well known that the incidence and prevalence of asthma is higher among women than men [17]. In addition, the number of adult individuals affected with food allergy is greater in females than males [18], and it has been suggested that female gender could be associated with an increased risk of anaphylaxis [19]. Therefore, having more females in the control group, who would be expected to have stronger allergic responses, was actually

associated with negative skin test results and undetectable IgE to the purified allergens.

The question of whether parasites protect from or promote allergic diseases and asthma remains. In human subjects, outcomes of studies with experimental infections with live parasites using either the pig whipworm *Trichuris suis* (eggs administered orally) or the human hookworm *Necator americanus* have been disappointing [1, 20, 21]. A randomized controlled trial investigated the efficacy of *T. suis* for the treatment of allergic rhinitis in Denmark but showed no clinical benefit. Likewise, a small randomized controlled trial in patients with asthma showed no significant benefit of hookworm infection on clinical symptoms, bronchial responsiveness, or SPT reactivity [1]. It is possible that helminth infections would do little to revert symptoms once the allergic reaction is established, and therefore prospective studies starting during pregnancy and early life would be more appropriate [3]. Ethical issues regarding the prompt treatment of parasite infections as soon as they are diagnosed, which is the best practice to adopt, should be considered in study designs due to the harmful nature of these infections, particularly when involving young children. Ultimately, therapeutic use of parasite molecules with the potential to trigger a regulatory response could lead to the downregulation of allergic responses and asthma control [1, 6], but these studies are still preliminary. Our results are in keeping with findings of studies in Colombia [7] and suggest that IgE responses to tropomyosin from *A. lumbricoides*, and perhaps to other parasite molecules sharing homologs with inhalant and food allergens [1, 22], may enhance reactivity to homologous allergens upon exposure by inhalation or ingestion, either promoting allergic reactions and asthma or increasing the severity of these clinical conditions. Therefore, strong efforts to eradicate these parasite infections should be a priority worldwide.

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Statement of Ethics

The study was approved by the ethics committee of our institution, under the protocol No. 942.956, and all patients or their guardians gave their informed written consent to participate in the study.

Disclosure Statement

The authors have no relevant conflicts of interest to declare.

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Author Contributions

A.C.A.F. Sousa-Santos coordinated the clinical aspects of the study, carried out patient recruitment, performed the skin tests, conducted the acquisition of patients' data, and prepared the first draft of the manuscript. A.B.R. Santos produced recombinant *P. americana* and *A. lumbricoides* tropomyosins in our laboratory, and also prepared the allergens for skin testing. M.C.R. Barbosa performed the total IgE measurements and the ELISA experiments. D.C. Aragon conducted the statistical analysis and contributed to data interpretation. Valeria S.F. Sales, A.S. Moreno, and L.K. Arruda designed the study, analyzed the data, and revised the manuscript to its final version. A.S. Moreno also performed the ImmunoCAP-ISAC experiments. All authors contributed to the interpretation of the data, revised the manuscript, and approved the final version.

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