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Allergy in the tropics: the impact of cross-reactivity between mites and ascaris

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1. ABSTRACT

Allergic diseases and nematode infections such as ascariasis are important health problems in underdeveloped tropical countries. The co-exposure to *Ascaris lumbricoides* and the domestic mites *Blomia tropicalis* and *Dermatophagoides pteronyssinus* induces a strong Th2 and immunomodulatory responses that can modify the natural history of both diseases. An associate phenomenon of these particular environmental conditions is cross reactivity between mite and Ascaris allergens. We demonstrated a high IgE cross reactivity between the allergenic extracts from both sources and that several already known allergens like tropomyosin and glutathione-s-tranferases are involved. Although this cross reactive antibody response has not been completely analyzed, there are clinical and experimental evidences suggesting that it could be an important component of the complex interactions between ascariasis and mite allergy. For example, it may affect the specificity of serological IgE tests for diagnosing both ascariasis and allergic diseases and, in consequence, the results of epidemiological surveys evaluating the predisposing or protecting role of ascariasis on allergy. In this review we discuss the potential role of cross reactivity on several aspects of allergy in the tropics that have been the matter of a number of investigations, some of them with controversial results.

2. INTRODUCTION

Allergic diseases are worldwide distributed but some particularities in the pathogenesis and prevalence are evident mainly due to the type of allergens and the genetic background of the population. Their aetiology is unknown and any information from the analysis of contrasting regions could be useful to decipher the origin of these public health problems. In the tropics allergies are strongly influenced by mite allergens and parasitic diseases. The effects of these particular environmental conditions are theoretically interesting and have important clinical repercussions. For unknown reasons, domestic mites, for example *Dermatophagoides pteronyssinus* and *Blomia tropicalis* and intestinal nematodes like *Ascaris lumbricoides* are potent inductors of IgE synthesis and raised an immune response characterized by a strong Th2 response including eosinophilia.

Several studies have consistently demonstrated that mite allergens are an important allergenic source in tropical regions (1-6), where warm temperatures and high humidity associated with lack of seasons facilitate the proliferation of around six species of clinically important mites, being the population perennially exposed to their allergens (7), largely from *D. pteronyssinus* and *B. tropicalis* the most prevalent mites in home dust (8, 9).

Most underdeveloped countries, where nematode infections range from hypo-endemic to hyper-endemic, are located in the tropics. The poor sanitary conditions promote parasitic exposure early in life. Although several nematodes are common in these environments, *A. lumbricoides* is the most prevalent affecting ~1.5 billion people worldwide (10). This parasite infects humans by oral contamination with embryonated eggs and contacts the immune system during its larval migratory phase through the intestine, liver, systemic circulation and lungs (11).

Some aspects of the relationship between allergic and parasitic diseases have raised the interest of clinical and basic researchers, among them: a) the common Th2 immunological mechanisms involved in their pathogenesis; b) the influence of allergy in the defence against parasitic diseases and the influence of parasitic diseases on allergy inception and clinical evolution; c) the common genetic mechanisms underlying the IgE responses in both diseases and, d) the effect of parasitic infections on total IgE levels and skin test with allergens.

The effect of an early co-exposure to mite and nematode allergens on the pathogenesis of both diseases is still unknown but could potentiate or suppress the allergic immune response by several mechanisms (12, 13). What is known is that it induces high levels of total IgE and specific IgE to a number of molecules and there is a cross reactive antibody responses involving protein epitopes (14). Besides, the role of *A. lumbricoides* as a risk factor for asthma and allergy has been largely studied and the results are still controversial (15). In some epidemiologic surveys the infection is a predisposing factor for allergy and asthma (16-21) while in others is protective (22-25). Although several reasons can explain this variability, cross-reactivity between Ascaris and other invertebrates including mites could confound the results of some of these investigations, especially those epidemiological approaches that use *Ascaris spp.* extracts to quantify specific IgE as a marker of infection. In this review we analyze some epidemiologic and basic aspects of allergic diseases in the tropics and the potential impact of cross reactivity between Ascaris and domestic mites in their pathogenesis and diagnosis.

3. ALLERGIC DISEASES IN TROPICAL REGIONS

Although it is believed that allergic diseases are infrequent in underdeveloped countries, the fact is that they are very prevalent in urbanized areas of middle-income (26). The prevalence of asthma and rhinitis has been evaluated in those countries demonstrating that these problems are as frequent as in industrialized societies (27-30). Urbanization can increase the prevalence of asthma and allergy in developing countries (31), however, the specific environmental risk factors leading to remarkable differences in allergy prevalence between rural and urban communities remain unclear.

There is no evidence that allergic diseases have unusual clinical symptoms in the tropics but sensitizer allergens come mainly from domestic mites, being pollens, cockroach, pets and mold allergens less clinically important (6, 32). Also, relevant food allergens are also expected to be different. Interestingly, the progression of allergic symptoms during infancy seems to be different to the "allergic march" observed in European countries. Respiratory symptoms like early wheezing, allergic rhinitis and asthma are the most common manifestations, being atopic dermatitis less frequent. For example, in Colombia, among children from 1 to 4 years old, the prevalence of wheeze ever is 40.1% with 7.6% requiring hospitalization in the last 12 months while the prevalence of atopic dermatitis is 4.9% (28).

As mentioned, an interesting feature of many tropical regions is the endemic presence of helminthes and other parasites, however, the extent of the influence of those infections and mite co-exposure on the prevalence, age of inception, severity and frequency of allergic episodes is not known, but as we will analyze later in this review, there are theoretical possibilities that deserve further investigations.

4. IgE CROSS REACTIVITY BETWEEN DOMESTIC MITES AND ASCARIS

Cross reactivity is a frequent feature of the adaptative immune response involving antibodies or T lymphocyte receptors directed to diverse molecules (antigens or allergens) and resulting in different biological or clinical effects. Cross reactivity occurs when the immune response elicited to one epitope also recognizes identical or similar epitopes in other molecules. This phenomenon can be beneficial for immunity against infectious agents but could be highly risky in cases of allergy or autoimmunity. In clinical practice the term is used broadly to cover those reactions to different sources of allergens (e.g. kiwi and banana) independently of the molecular basis of the reaction. The allergen that is supposed to induce the original allergic responses is named primary sensitizer. The other allergens are considered cross reacting allergens. There are several clinical and laboratory criteria to classify an allergic reaction as a cross reactive immune responses (33).

The clinical relevance of allergic cross-reactivity have been well documented for foods, pollens, mites and other allergenic sources (34), but its experimental study in mite and nematode allergens is just at the very beginning; as a consequence the clinical and epidemiological impact is unknown. Cross reactivity depends of amino-acid sequence and conformational structure of the molecules involved, that is the reason why it is more frequent (but not exclusively) among phylogenetically related allergens. Ascaris and mites are close related invertebrates belonging to the Super Phylum Ecdyzozoa and expected to share several allergens. Using bioinformatics tools we analyzed the amino acid sequence identity between some of their proteins and predicted potential cross reactive epitopes in some allergens, which are currently under experimental analysis. Independently of which source is the primary sensitizer, among inhabitants of the tropics, allergenic stimuli derived from a persistent inhalation of high concentrations of mite allergens and infections with *A.*

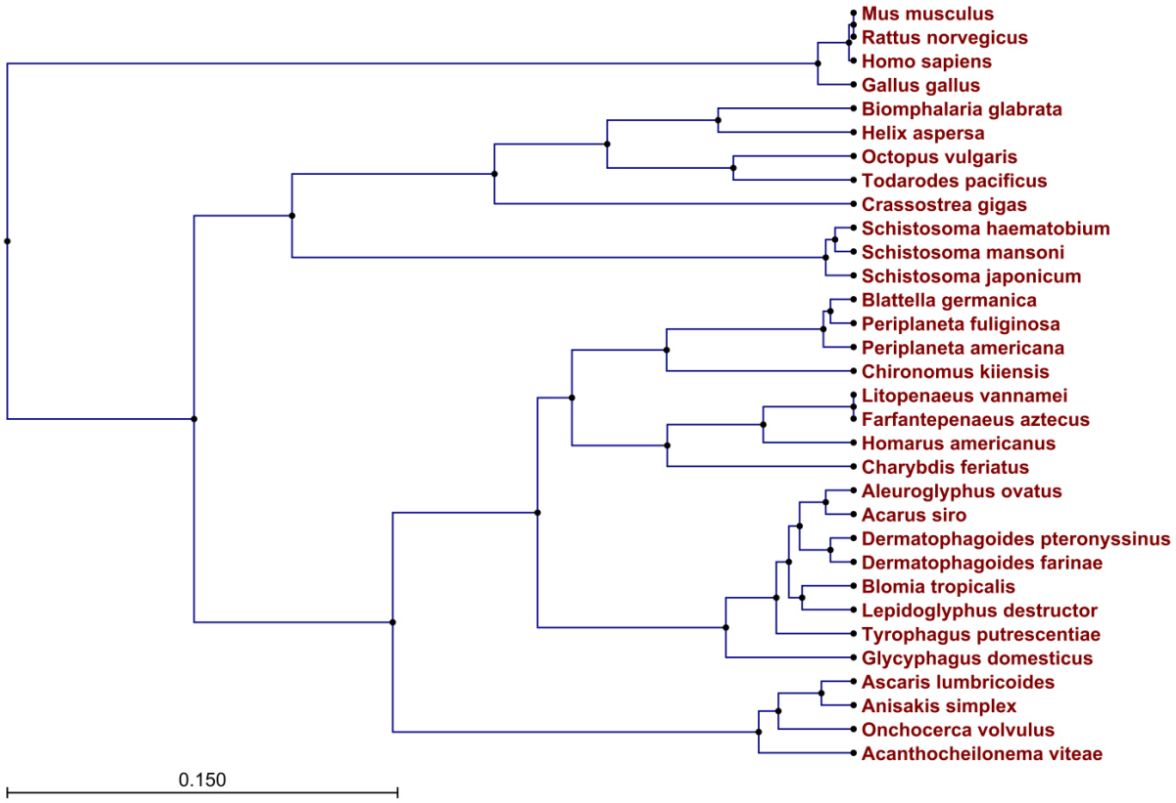

Figure 1. Phylogenetic tree of some species based on the identities of tropomyosin sequences. The UPGMA algorithm and CLC workbench 5.6.1 were used. Tropomyosin sequences of Introverta (grouping nematodes) and Arthropoda (grouping insects, crustaceans and arachnids) are closely related and derived from the common ancestor Ecdysozoa.

lumbricoides could generate a particular immune response that involves cross reactivity in both ways.

We performed dose-response ELISA and immunoblotting inhibition studies employing extracts of *B. tropicalis*, *D. pteronyssinus* and *A. suum*, demonstrating that there is a high degree of cross reactivity between those sources including protein IgE-epitopes and the pan-allergen tropomyosin (14, 35). Our experiments strongly suggest that mites are the primary sensitizer; using sera from asthmatic patients we found that clinically relevant allergens such as tropomyosin and glutathione tranferases are involved. In contrast to the opinion that cross-reactivity between parasites and house dust mites in tropical regions is irrelevant (36), we consider that is very important and postulate that the high prevalence of specific IgE antibodies to mites detected in tropical populations could be influenced by cross-reactivity with *Ascaris ssp.* allergens, and this partially explains the high prevalence of allergy observed in urban areas of the tropics even in places with poor hygiene conditions.

5. THE ALLERGENIC COMPOSITION OF ASCARIS AND MITES

The antigenic composition of *Ascaris spp*. has been extensively investigated and some molecules (e.g. As14, As16, As24, As37, PAS-1) have been analyzed (12, 37). Antigens like PAS-1 have immunoregulatory properties; As24 and As16 confer protection from migration of *A. suum* larvae through the lungs as demonstrated in experimental vaccination models (37, 38). However, our knowledge on the allergenic composition of nematodes is still very limited. So far, the IUIS Allergen Nomenclature Sub-Committee has recognized several allergens from *Anisakis simplex* and the allergens ABA-1 (Asc s 1) and tropomyosin (Asc l 3) from *Ascaris ssp*. Because of the close relatedness of the nematodes *Anisakis simplex* and *A. lumbricoides*, it is possible that previously described allergens in *A. simplex* (e.g. tropomyosin, cysteine and serine protease inhibitors, paramyosin, etc) also exists in *A. lumbricoides* and cross-react with allergens from other invertebrates. Since almost all allergens from domestic mites have been identified, it is now possible to study their cross-reactivities with Ascaris allergens. Due to its phylogenetic relationships, it is expected that some mite allergens could also be allergens in nematodes. Indeed, cross reactivity between *A. simplex* and *D. pteronyssinus* has been described (39). Since our preliminary results suggest that mite tropomyosin and GST have cross reacting counterparts in Ascaris extract, we now discuss the current evidence supporting these molecules as potentially relevant cross-reacting allergens between nematodes and mites.

5.1. Tropomyosin, an invertebrate pan-allergen

Tropomyosin belongs to a family of phylogenetically conserved proteins of eukaryotes (Figure

1) and is considered a pan-allergen because of its extensive cross reactivity among different invertebrate species (40, 41). There are several isoforms resulting from alternative splicing in different tissues. In muscle cells, tropomyosin is essential for muscular contraction while in non-muscle cells acts as a mechanic support of the cell membrane and intracellular transportation of molecules. The amino acid sequence has regions of high percentage of identity among species in the repetitions of heptapeptides that determine its coiled coil structure with two parallel alpha helices. Although most amino acids are conserved (especially within actin-binding motifs) some stretches of sequence differ enough between vertebrate and invertebrate tropomyosins to induce IgE antibody responses in mammals (42). Tropomyosin is widely recognized as the major shrimp allergen (43, 44) and is the most important allergen in other species of crustaceans, mollusks and cephalopods (45, 46); in addition is a potent inhaled allergen from cockroach and mites and a known target for IgE antibodies during infection with nematodes (47-49). In mites, tropomyosins have been classified as group 10 allergens, Der p 10, Der f 10, Blo t 10, Lep d 10 and Tyr p 10 (50-53). In crustaceans and mollusks they belong to group 1 allergens (e.g. Pen a 1, Met e 1, Pen i 1, Hom a 1, Pan s 1, Cha f 1, Cra g 1, Tur c 1, Tod p 1); to group 7 in cockroach (Bla g 7, Per a 7 and Per f 7) (54, 55) and group 3 (Ani s 3 and Asc l 3) in nematodes.

Invertebrate tropomyosins are highly allergenic for mammals and it has been proposed that ancestral point mutations on current non-allergenic tropomyosin abolished their IgE binding capacity (42, 56, 57). It is also possible that invertebrate tropomyosins remain as longer peptide fragments after digestion with Pepsin A compared with tropomyosins from chicken, rabbit or fish, which could make them more allergenic (58). Clinically relevant tropomyosins cross reactivities with crustaceans and mites have been reported in patients allergic to shrimp (59). Shrimp consumption by mite allergic patients can trigger severe asthma symptoms and patients receiving immunotherapy with mite extracts can become *de novo* sensitized with mite tropomyosins and react to shrimp. Moreover, mite allergic patients not exposed to shrimp exhibit positive IgE against the shrimp tropomyosin Pen a 1 (60).

Although these cross reactivities have been extensively analyzed (61-64), there are few studies evaluating that between tropomyosins from mites and nematodes. This is of particular interest in tropical regions, where intestinal helminthes exposure represents an additional factor in the relationship between tropomyosins from invertebrates. There are evidences supporting the existence of this cross reactivity. *A. simplex* is a well-known allergenic source after ingestion of infected fishes. Pascual *et al*, described that it has important cross-reactivity with midges and cockroach (65) and Johansson *et al*, showed that *A. simplex* extract exhibit cross-reactivity with four different species of mites (*Acarus siro, Lepidoglyphus destructor, Tyrophagus putrescentiae and D. pteronyssinus*) including a 36 kDa allergen supposed to be tropomyosin (39). In *A. simplex*, tropomyosin was cloned and expressed as a 284 amino acid protein (Ani s 3) with 75% identity with mite tropomyosins and proposed as trigger of several clinical manifestations including urticaria, angioedema and gastrointestinal symptoms (66).

Santos *et al.* cloned the tropomyosin from *A. lumbricoides* and described a strong correlation between IgE levels to Ascaris and cockroach tropomyosins (49). The amino acid sequence identity of this molecule with mite tropomyosins is 73% and share several regions predicted as IgE binding epitopes in shrimp tropomyosin. Using cross inhibition ELISA and immunoblotting studies we demonstrated the allergenic cross-reactivity between Blo t 10 and Ascaris tropomyosin using sera from asthmatic mite-allergic patients (14, 35). The clinical relevance of these findings is currently under study; although there are evidences suggesting that cross-reactivity between mites and Ascaris tropomyosins could be important (40). Interestingly, in under-developed countries from Africa and South America where parasitic infections are frequent, IgE sensitization to cockroach and mite tropomyosins is observed in more than 50% of the population (67), being quite far more frequent than 5-16% of sensitization in wealthy regions (50, 68). Further studies are necessary to evaluate at the population level, if the IgE response to nematode tropomyosins are more directed to cross-reactive epitopes or species-specific epitopes (69)

There is other interesting side of cross reactivity: considering that IgE response to nematode tropomyosins has been related with protection to Ascaris infection and the molecule is a candidate for vaccination purposes (48), studies trying to induce nematode protection by vaccinating with this molecule should take into consideration the potential risks of allergy sensitization with other sources. In fact, an immunodominant epitope (AQLLAEEADRKYD) recognized by human IgG antibodies in *Onchocerca volvulus* tropomyosin (Ov-TMY-1) which protects from high filarial densities in skin coincides with an epitope recognized by patients with shellfish allergy (47).

5.2. The Glutathione S Transferase Superfamily

Glutathione-s-tranferases (EC 2.5.1.18) are multifunctional enzymes representing an important system for detoxification in eukaryotes by addition of reduced glutathione (GSH) to endobiotics, xenobiotics and electrophilic substrates. They exist as cytosolic, transmembrane and microsomal isoenzymes, being cytosolic GSTs encoded by related gene families while membrane and microsomal GSTs are encoded by single genes. Based on their sequence similarity, immunological cross-reactivity and substrate specificity, the GSTs have been grouped into species-independent classes of isoenzymes (namely, Alpha, Mu, Pi, Theta, Sigma, Kappa, Phi and Zeta).

In worms these enzymes have important functions as part of a type II detoxification system essential for parasite survival, neutralization of oxygen reactive species and metabolism of environmental substances, including antihelminths and nutrients. Interestingly, nematodes have GSTs classes that are not represented in

mammals, which exhibit differences in function, abundance and biochemical activity. In filarial worms some extracellular glycosylated isoenzymes like Ov-GST1 are involved in the synthesis of prostanoids products that could modulate the host immune system (70). The cytosolic isoforms like Ov-GST2 accounts for the 0.1% of the total protein content of the adult worm and is confined to the intracellular compartment (71).

Some GSTs from mites (72), cockroach (73), arthropods and molds are allergenic (74). Although the primary sequence identity is low, they harbor conserved amino-acids in the N-terminal domains that determine similarities in their tertiary structure, and result in homodimers or heterodimers with a conserved structural fold. These similarities in protein topology could explain why being an ancient and diversified species-independent protein family are also cross-reactive allergens in some sources like molds (75) and invertebrates (72).

The clinical relevance of GST allergens from cockroach and mites is supported by several studies describing high percentages of IgE sensitization. Recently a GST from *D. pteronyssinus* (Der p 8) was cloned and identified as a basic isoform (pI = 8.5) containing six polymorphic residues and recognized by IgE antibodies from 84% of Taiwanese asthmatics tested. Inhibition studies with cockroach extract and native purified GST, showed that Der p 8 is cross reactive with cockroach GST (72). It is not clear if some GSTs could be more allergenic but in cockroach, different classes have been described as allergens being Bla g 5 a sigma class, while a recently described IgE binding isoform (BgGSTD1) exhibit 45-60% of sequence homology with delta class and only 14% with Bla g 5 (76), showing 17.9% of IgE reactivity in cockroach allergic patients (77).

The Glutathione-s-transferase of *A. suum* (rAsGST1) was cloned by Liebau *et al* (78), as a protein of 26 kDa and is detected in high concentrations in the gut of this nematode. This is an intracellular protein without suspected post-translational modifications and active as a dimer. The three dimensional structure models showed that is more related to Pi class of GSTs (79). It is unknown if this molecule is allergenic and we are currently investigating this issue; we found in the Ascaris extract the IgE recognition to a 23 kDa allergenic component that was inhibited by mite extracts and confirmed as GST by mass spectrometry (14). In addition, we observed that 10% of our allergic patients have specific IgE antibodies to *Schistosoma japonicum* GST (80). However since this parasite is not found in our region it is possible that in this study we only detected cross-reacting antibodies identifying epitopes shared with Ascaris GST.

The number of allergens of Ascaris seems to extend beyond tropomyosin and GST because the sera from asthmatic patients detected at least 12 IgE binding components in the extract (14). The identification of those molecules and the important question of which of them are cross reactive with mite allergens and which are species-specific, are currently under investigation in our laboratory.

6. THE INFLUENCE OF PARASITE INFECTIONS ON ALLERGIC DISEASES: EPIDEMIOLOGICAL STUDIES

The relationships between Ascaris infection and allergic diseases have been a matter of investigation of scientists and clinicians covering a broad number of disciplines. For example, it is very popular, although not necessarily correct, the evolutionary notion that allergic diseases are the result of a remaining immune response protecting against parasite infections, although it has been found that atopic subjects trend to be more resistant to these parasitic diseases (81). The shared components and the effects of interaction between nematode infections and allergic response have been explored using basic-experimental and epidemiological approaches, the latter with more controversial results (15).

Different criteria for diagnosing Ascaris infection have been employed in population surveys. One common marker is the specific IgE antibodies to the nematode extract (21). It has been proposed that this serological approach is more sensitive and specific than the detection of the parasite in faeces (82). However, this diagnostic tool still has the problem of the cross reactivity of Ascaris with other nematodes; and the new evidence of cross reactivity with domestic mites complicates the scenery because mite allergens are the more important environmental risk factor for asthma and atopy in the tropics. There are some indirect epidemiological data supporting the effect of this cross reactivity at the population level.

A high prevalence of Ascaris IgE seropositivity has been observed in populations from developed countries where Ascaris infection is not expected to be endemic. For example positive IgE to Ascaris was detected in 15-20% of school children in the former East Germany (21). Among farmer children from Austria, Germany and Switzerland, IgG to the Ascaris extract was positive in 33.9% of children (83). Other result commonly observed is a high frequency of Ascaris sensitization with a low prevalence of eggs in faeces. In Cape Town the prevalence of Ascaris infection (as diagnosed by parasite egg counts) was 14.8% while positive IgE levels to Ascaris were detected in 49.9% of the population (20). In Costa Rica, although Ascaris eggs were not found in faeces, 38.9% of the subjects had positive IgE antibodies to *A. lumbricoides* (18). According with the ecology of ascariasis these findings may be due to acquired resistance, but they also suggest cross-reactive non-parasitic sources stimulating the specific IgE synthesis.

Therefore, this cross reactivity should be considered as a potential confounding factor when studying the effect of ascariasis as risk factor for asthma and allergy in the tropics. We have analyzed several publications about this issue and found that not all authors adjust by this confounding, and when this is done some associations are maintained and other disappeared (Table 1)

In a survey of 2164 subjects between 8 and 18 years in Anqing China, infection with *A. lumbricoides* conferred a higher risk for asthma and the number of skin

Table 1. Some studies suggesting a predisposing role of Ascaris infection on allergic diseases and atopy across different populations, considering OR before and after adjusting by mite sensitization

Population	n (Age)	Characteristics	Prevalence of Ascaris in stool samples	Prevalence of positive Anti-Ascaris IgE		Associated Symptoms	OR (95% CI, p value)	OR (95% CI, p value) adjusting by mite sensitization	Ref
				All	In mite allergics				
Zerbst and Hettstedt; Former East Germany	668 (5-14)	German schoolchildren Low prevalence of nematode infections	Not evaluated	15.7%	52.7%	Positive IgE to Ascaris and allergic sensitization	3.65 (2.86 - 4.67, p < 0.001)	NR	(21)
Anqing, China	2164 (8-18)	Rural communities	12.2%	Not evaluated		Positive eggs in stool examination and increased risk of asthma	2.26 (1.58-3.23, p < 0.001)	NR	(16)
Cape Town, South Africa	359 (6-14)	Randomly selected sample Urban, poor sanitary conditions	14.8%	47.9%	68.8%	Positive IgE to Ascaris and HDM sensitization	3.81 (1.76-8.26, p < 0.05)	NR	(20)
Matlab, Bangladesh	402 (5)	219 (wheezers) 183 (controls) Rural population, highly endemic for Ascaris	90%	92.8%	NR	Positive IgE to Ascaris and current wheezing	1.40 (1.2-1.64, p < 0.001)	1.31 (1.0-1.60, p = 0.007)* 1.33 (1.0-1.77, p = 0.05)**	(17)
						Positive IgE to Dp and current wheezing	1.23 (1.0-1.47, p = 0.01)	1.25 (0.9-1.68, p = 0.13) adjusted by Anti-Ascaris IgE	
Costa Rica	439 (6-14)	Asthmatics Urban, low prevalence of Ascariasis	0%	38.9%	94.2%	Positive IgE to Ascaris and skin reactivity to > 1 allergen	5.15 (2.3-11.2, p < 0.001)	0.62 (0.19-1.9, p = 0.41)	(18)

*Adjusted by Anti-Dp among other covariates, **Adjusted by anti-Dp IgE and total IgE among other covariates. HDM, house dust mite, NR, No reported

tests positive to aeroallergens. Unfortunately the authors did not reported adjustments by positive skin test to individual allergens like cockroach or house dust mites (16). One study performed in Costa Rica showed that specific IgE against *A. lumbricoides* extract is a risk factor for the number of positive skin test and bronchial hyper reactivity (18). In a logistic regression analysis the authors found that the significance of this association disappeared when correcting by specific IgE to mites and cockroach.

7. THE INFLUENCE OF PARASITE INFECTIONS ON ALLERGIC DISEASES: EXPERIMENTAL ANALYSIS

Nematodes are potent inductors of IgE synthesis and characteristically raised a strong Th2 response, synthesis of IL-4 and IL-5, eosinophilia and mucus hyper secretion (11). This response is evoked by somatic and excretory/secretory antigens during larvae molting and confers protective mechanisms allowing to expel the adult parasites from intestine and to resist re-infections. Immune recognition of parasite occurs at different tissues, being mesenteric lymph node antigen presenting cells in early contact with its antigens after intestinal penetration of larvae and its destruction in liver granulomas. Surviving larvae migrates to lungs generating an inflammatory infiltrate in the airways, mainly severe peri-alveolar eosinophilia and induction of specific antibodies. Humoral response against egg and larvae includes IgM, IgG, and IgE; the production of high levels of polyclonal IgE and specific IgE against Ascaris allergens is a hallmark of this infection in humans. Most of the IgE is induced by B cell activation in an IL-4 dependent fashion and by mitogenic substances in the body fluid of Ascaris; it is believed that only a little proportion of those antibodies are targeting parasite specific allergens (84).

Many features of the aforementioned anti-Ascaris immunity are shared by the allergic response to environmental allergens. Epidemiological descriptions of positive associations between Ascaris infections and allergic sensitization can be biologically explained by a systemic Th2 enhancement that induces an allergic polarization to bystander antigens. According to this idea the invasive larvae not only induces allergic inflammation but may promote allergic responses directed to non-parasite

allergens, such as aeroallergens (85). To date little experimental evidence has supported this concept. Earlier studies described that antigens of *A. suum* potentiate "reaginic" response to OVA (86, 87). Also, Ascaris pseudocelomic body fluid and the purified allergen ABA-1, prolonged the response to ovoalbumin as third-party allergen but they did not enhance the IgE levels to OVA (88). There is also evidence that unsolved components in the parasite body fluid promote a Th2 response and are adjuvants for specific IgE production to some parasitic allergens like ABA-1 (89). In other study, co-administration of hen egg lysozyme with the excretory/secretory products from the related nematode *Nippostrongylus brasiliensis* results in generation of egg lyzozyme-specific lymphocyte proliferation, IL-4 release and IgG_1 antibody responses, supporting the role of some nematode proteins as adjuvant for third-party antigens (90).

It has been proposed that mild or intermittent cycles of parasite infections increase allergic reactivity and heavy, chronic infections suppress several components of adaptative immunity, including the allergic response. However, loads of parasite, co-exposure to other environmental factors and atopy susceptibility could modify the effects of Ascaris infection in different communities (91). The regulatory network associated with chronic helminth infections has been largely analyzed, finding that parasites produces several substances that prevent strong host response, facilitate immune evasion, long-time survival and prevent tissue damage. These mechanisms are thought to reduce responses to non-helminthes antigens, like allergens possibly leading to lower prevalence of allergies in subjects that are chronically infected with helminthes (92). The biological pathways underlying this protective effect of Ascaris infection on allergy is unknown, but there are interesting findings in animal models suggesting that this modulation involves innate recognition, antigen presentation, T cell differentiation, antibody production and apoptosis. It is worth noting that immunomodulation by parasites could vary depending of the organism, life cycle and degree of infection.

There are evidences suggesting that the first contact of parasite antigens with the innate immune system determines the evolution of the response to Th2 profile or T regulatory. Helminths molecules modulate dendritic cells and Toll-Like Receptors signaling, including TLR2, 3 and 4 (92). Liso-phosphatidylserine from *Schistosoma mansoni* binds TLR2 (92) and dsRNA of this nematode binds TLR3 (93), inducing Th1 responses, nitric oxide production and activation of proinflammatory cytokines (92). *A. lumbricoides* also contains lipids that stimulate TLR2 inducing the development of Treg cells although the involved structures remain to be characterized (94). Phosphorylcholine-containing glycosphingolipids from *A. suum* significantly reduced proliferation of splenic B cells and inhibited IL-12 p40 production by peritoneal macrophages (95). In humans, the role of innate immunity receptors in the response to gastrointestinal nematodes was suggested in children from Tanzania where worminess was positively associated with production of TNFα in response to TLR4 stimulation with LPS (96).

Allergic suppression by nematodes is also mediated by several mechanisms of the adaptative immunity, including regulatory T cells and immunosuppressive cytokines. Infection with *Heligmosomoides polygyrus* suppresses lung inflammation in mice. This protective effect is adoptively transferred by mesenteric lymph node cells containing elevated numbers expressing a regulatory phenotype (CD4+ CD25+ Foxp3+) and a strong TGFβ1 and IL-10 production (13). In *A. suum* extract this immunosuppressive effect is primarily related to its property of down-regulating the Ag-presenting capacity of dendritic cells via an IL-10-mediated mechanism (97). The same mechanisms are supposed to occur in humans since African children under conditions of hyper endemic exposure to *A. lumbricoides* and *Trichuris trichiura*, secrete more IL-10 and TGFβ1 compared with conditions of mesoendemic exposure (98). Ascaris proteins have shown to suppress the allergic response to bystander antigens like ovoalbumin (89, 99). Interestingly, the pseudocelomic fluid of *A. suum* also suppress the allergic response to ragweed in a IL-10 deficient mice, suggesting that IL-10-independent mechanisms also participates in this immunoregulation, possibly by interference with dendritic cells function (100). In agreement with this, IL-10 production was similar among infected and non-infected subjects in a population under endemic exposure in Ecuador (101) which exhibit a significant reduction of skin tests responses to aeroallergens in parasited patients.

Although the mechanisms are still unknown, immunomodulation resulting from exposure to Ascaris body fluid is limited to the induction phase of allergen sensitization but not if co-administered during the effector response (89). Also, immunosuppressive and stimulatory effects are due to distinct components of *A. suum* adult worms. For example high molecular mass components strongly suppress the humoral and cellular immune responses while low molecular weight components induce antibody production (102).

Since most studies showing a suppression of the immune response by parasite infections are related with heavy and chronic infections, it is theoretically possible that in circumstances of infections with low intensity, the simultaneous exposure to both species specific and cross reacting allergens from mites and Ascaris allergens, result in a strong IgE responses against molecules from those sources. As the exposure to mite allergens is perennial, it is possible that cross reacting allergens from mites stimulates and sustain specific IgE response to some Ascaris allergens.

8. MITES AND NEMATODE CO-EXPOSURE: GENETICS AND POSSIBLE INFLUENCES OF CROSS REACTIVITY IN THE ALLERGIC RESPONSE

In endemic regions *A. lumbricoides* can infect children early in life (around 3 months of age) but the consequences in allergy pathogenesis are not clear. Before discussing how Ascaris infections could influence allergy inception and evolution it is important to consider the role

of genetics in the generation of specific allergic responses. Immunity to nematodes is genetically controlled (103) and the genetic background could predispose some individuals to have a more prominent Th2 response (104, 105). Therefore, under nematode-stimuli some subjects can develop a strong Th2 response, possibly useful to eliminate the parasite (106) but adverse to regulate the immune response to other environmental antigens. In humans, humoral response to Ascaris includes IgG and IgE antibodies (104); both isotypes are important as defence mechanisms against the parasite, being IgE and IgG_1 related with protective responses and IgG_4 with susceptibility to infection. The Major Histocompatibility Complex can restrict the type of epitope that is recognized, possibly making some individuals able to present nematode specific antigens (like ABA-1) and others prone to present epitopes from cross reactive allergens like tropomyosin. Recent studies in our laboratory have suggested that some genes controlling the IgE responses to Ascaris extract and ABA-1 allergen have no influence on specific IgE to mite allergens (107). These genetic aspects could explain why in Ascaris endemic communities healthy non-allergic subjects produce IgE antibodies to Ascaris without mite sensitization. In this way of reasoning, besides exposure variability, individual susceptibility has a role to define if subjects co-exposed to Ascaris and mite allergens become IgE sensitized to nematode specific-antigens, to mite specific-allergens or both.

Previous studies have shown that IgE response to *A. simplex* decays after six months of allergen contact (108). We hypothesize that if nematode infections are endemic and people are perennially exposed to mites, cross-reacting allergens provide a permanent boosting for memory lymphocyte clones. At the population level, the intensity of infection with *A. lumbricoides* decreases with age after a peak within the first decade of life in high-intensity areas but IgE antibodies remains long-lasting. Exposure to cross-reacting allergens (e.g. by inhalation or a chronic exposure to nematodes) could promote B cell stimulation and sustain the synthesis of high specific IgE levels by plasma cells (109). These effects could influence not only the inception of allergic diseases but also the clinical evolution of asthma; for example, in some susceptible subjects may contribute to reduce the threshold for triggering allergic reactions and the perpetuation of inflammation in the airways. The less evident possibility that, under appropriate conditions, cross-reactivity of Ascaris with mites could reduce the response to particular allergens cannot be ruled out.

9. IMPACT OF CROSS REACTIVITY IN THE DIAGNOSIS OF ASCARIASIS: ABA-1 AS A NEMATODE SPECIFIC MARKER OF INFECTION

Traditionally the diagnosis of Ascaris infection has been done by detection of parasite eggs in stool samples. However, evaluation of *A. suum* infection in pigs showed that egg counts in faeces greatly underestimate the proportion of exposed pigs compared with anti-Ascaris IgG titration by ELISA (110). Similar findings were obtained in humans, where serodiagnosis of ascariasis, as detected by Ascaris positive IgE, exceeds in three fold the positive egg prevalence (82, 111). It is now well recognized that egg counting give important number of false negatives and available immunoassays could be more sensitive (112). In addition, as specific IgE antibodies to helminths remains for long time (113), they provide an additional advantage allowing the identification of previous contacts with Ascaris, even in egg-negative adolescent and adults.

The search for useful serological markers for diagnosing Ascaris infection has taken a long time (114). Some studies have evaluated the whole nematode extract (SAg) and others the pseudocelomic fluid or preparations of excretory/secretory antigens (E/SAg). As analyzed previously in this review, it is possible that a proportion of specific IgE to components of Ascaris extract (used by most commercial kits) are cross reacting antibodies elicited by mite allergens. Furthermore, serodiagnosis of ascariasis using extracts has the recognized problem of cross reactivity between several proteins of Ascaris and other nematodes (115-118). Then, it is necessary to employ more specific markers of Ascaris infection; different reagent alternatives are under investigation (119), one is the somatic antigen of 34 kDa (pSAg) purified from adult *A. lumbricoides* and recognized by IgG_4 antibodies of infected/exposed subjects (118). Other possibility is the use of the abundant allergen from the pseudocelomic fluid called ABA-1 (120).

ABA-1 (Asc s 1), a member of the Nematode Polyprotein Allergen/Antigens, is a very well characterized *Ascaris spp.* allergen (121-123). Several studies support that humoral immune response (IgG and IgE) to ABA-1 is associated with previous infection and natural immunity to Ascaris (112). In endemic populations, antibody isotype response to ABA-1 correlate with infection intensity being IgE associated with low infection levels and IgG_4 or seronegativity with high susceptibility to the infection (124). This protein of 15 kDa has only been found in nematodes, has fatty acid binding properties (125) is synthesized as a poly-protein and is in high levels in the pseudocelomic fluid of the parasite (121, 126). In our inhibition studies we found no cross-reactivity between ABA-1 and mite extracts (*D. pteronyssinus* and *B. tropicalis*), supporting its usefulness as a more specific marker of Ascaris infection. Since it is known that allergenic Fatty Acid Binding Proteins (FABPs) have been described in mites (e. g. Blo t 13, Der p 13, Der f 13), we also did inhibition experiments with the recombinant Blo t 13 allergen demonstrating that mite FABPs share no common epitopes with ABA-1 or other homolog allergen in Ascaris extracts (14). However, the sensibility and specificity analysis of serological tests with ABA-1 requires more studies, especially at the population level, because homologous molecules like gp15/400 ladder protein of filarial parasites (127) and TBA-1 from *Toxocara ssp* (128) could affect the usefulness of the assay. Current research in our laboratory evaluates these important aspects and also the possibility of cross reactivity between ABA-1 and other non-mite allergenic sources.

There is another important point that deserves attention and is the impact of cross reactivity in the diagnosis of mite allergy. It is well known that total IgE is not the best diagnostic parameter of allergy in the tropics because parasite infections increase serum levels of this immunoglobulin. Therefore, specific IgE to mites, the main sensitizers in allergic respiratory diseases, is considered an appropriate alternative. However, the existence of Ascaris cross reacting allergens could affect mite allergy diagnosis when using the whole extract as is routinely done *in vitro* and for skin testing. Of course, cross reactivity theoretically can bias any immunological test and has been extensively studied with aeroallergens, but the case of parasites could be quantitatively important because of their capacity to stimulate IgE response. Component resolved diagnosis could help to solve these problems.

10. CONCLUDING REMARKS AND PERSPECTIVES

Asthma and other allergic diseases are frequent and important health problems in several underdeveloped tropical countries, where environmental conditions allow the co-exposure of the population to mite and nematode antigens/allergens. The impact of the cross reactivity between these sources just begun to be evaluated with the empirical demonstration of this immunological phenomenon in humans, but a number of evidences strongly suggest that this is an important field of experimental and clinical allergology that needs more research. Among the potential impacts of this cross reactivity the most obvious is the reduction of specificity in serological diagnosis of Ascaris infection and mite allergy using whole extracts. However, the consequences could be more important for understanding the complex interactions that emerge in the pathogenesis of allergic diseases in tropical regions. Just to mention one of the numerous possibilities, we hypothesize that in some susceptible individuals, allergic sensitization could be enhanced by perennial boosting from invertebrate cross reacting allergens. To improve the investigations analyzing the role of ascariasis on atopic diseases, future epidemiological studies should consider the use of more specific markers of Ascaris infection, such as ABA-1, in addition to the whole nematode extract.

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