

Review Article

IgE cross-reactivity between *Ascaris lumbricoides* and mite allergens: possible influences on allergic sensitization and asthmaN. ACEVEDO^{1,2} & L. CARABALLO^{1,2}¹Institute for Immunological Research, University of Cartagena, Cartagena, Colombia, ²Foundation for the Development of Medical and Biological Sciences, Cartagena, Colombia

SUMMARY

Nematode infections such as Ascariasis are important health problems in underdeveloped countries, most of them located in the tropics where environmental conditions also promote the perennial co-exposure to high concentrations of domestic mite allergens. Allergic diseases are common, and most of patients with asthma exhibit a predominant and strong IgE sensitization to mites. It is unknown whether co-exposure to *Ascaris lumbricoides* and the domestic mites *Blomia tropicalis* and *Dermatophagoides pteronyssinus* potentiates Th2 responses and IgE sensitization, thereby modifying the natural history of allergy. Recently, we obtained experimental evidence of a high cross-reactivity between the allergenic extracts of these invertebrates, involving well-known allergens such as tropomyosin and glutathione transferases. There is indirect evidence suggesting that the clinical impact of these findings may be important. In this review, we discuss the potential role of this cross-reactivity on several aspects of allergy in the tropics that have been a focus of a number of investigations, some of them with controversial results.

Keywords allergens, *Ascaris lumbricoides*, asthma pathogenesis, *Blomia tropicalis*, cross-reactivity, *Dermatophagoides pteronyssinus*, IgE, the tropics

INTRODUCTION

Because of their close dependence on environmental factors, including allergens, allergies are expected to vary

between geographical zones. Probably for that reasons, the influence of helminth infections on the pathogenesis of allergic diseases has been under investigation for several years. Progressively, the research in this field has focused on specific issues and evaluated using different methodological approaches, the most relevant aspects being (i) the particularities of the Th2 mechanisms involved in the pathogenesis of parasite infections and allergy; (ii) the influence of allergy in the defence against parasitic diseases and the influence of parasitic diseases on allergy inception and clinical evolution; (iii) the genetic influences on IgE responses in both diseases; and (iv) the effect of parasitic infections on total IgE levels, skin tests with allergens and serological diagnosis of allergy (Figure 1).

Nematode infections are an important health problem in most underdeveloped countries, where, depending of the degree of social deprivation and exposure to parasites, the endemicity ranges from hypo-endemic to hyper-endemic. Although several helminths (such as *Trichura trichiuris*, *Ancylostoma duodenale* and *Schistosoma mansoni*) are common in these environments, *Ascaris lumbricoides* is one of the most prevalent, affecting about 1.5 billion people worldwide (1). Typically, poverty and bad sanitary conditions promote parasitic exposure early in life, and humans become infected by oral contamination with embryonated eggs. Immunity to *A. lumbricoides* is characterized by high levels of IgE synthesis, a strong Th2 response, eosinophilia and mucus hyper secretion; it is induced by somatic and excretory/secretory antigens of larvae and confers protection by expelling intestinal parasites and resisting reinfections (1,2).

Many features of anti-*Ascaris* immunity are shared by the allergic response to environmental allergens and, for still unknown mechanisms, domestic mites, like *Dermatophagoides pteronyssinus* and *Blomia tropicalis*, induce specific IgE synthesis and elicit a strong Th2 response including eosinophilia that contribute to the pathogenesis

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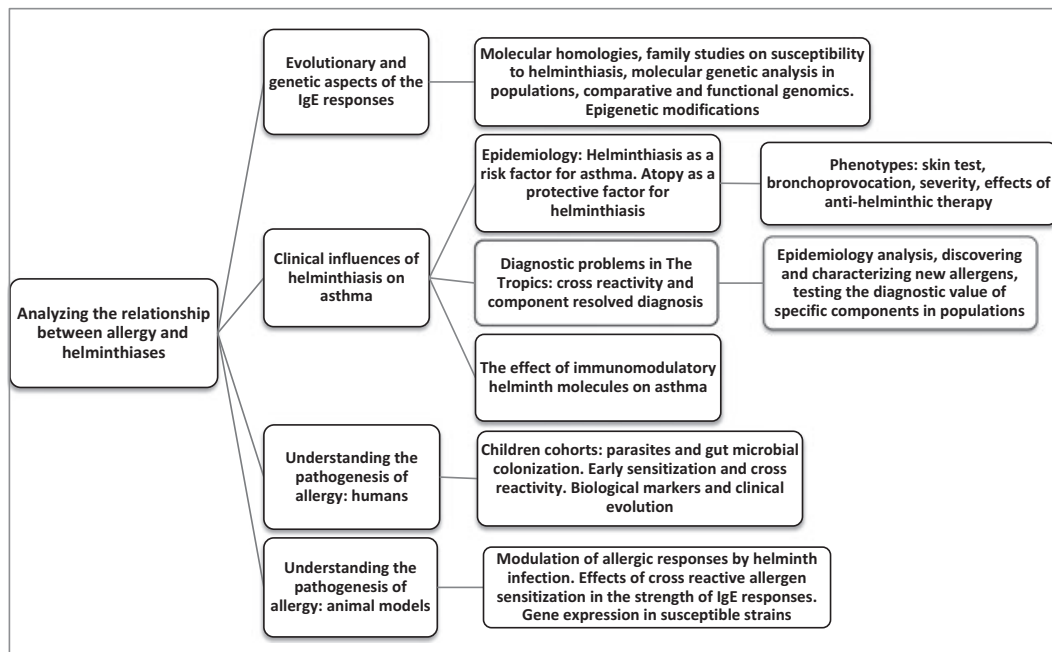


Figure 1 Different approaches for analysing the relationship between allergy and helminthiases.

of asthma and other allergic diseases. Because most under-developed countries are located in the tropics, populations are naturally co-exposed to both *A. lumbricoides* and mites, this situation therefore being of scientific and practical interest whether infections with this parasite predispose to atopy or protect from it.

Several studies have demonstrated that mites are important allergenic sources in tropical regions (3–8), where warm temperatures and high humidity permit the growth of around six clinically important species (9), mainly from *D. pteronyssinus* and *B. tropicalis* as the most abundant mites in house dust (10,11). The effect of an early co-exposure to mite and nematode allergens on the pathogenesis of allergies and helminth infections is unknown, but there are indications that it is able to either enhance or suppress the allergic immune response. The role of *A. lumbricoides* as a risk factor for asthma has been studied and the results are controversial, although has been associated with significantly enhanced likelihood of asthma in a systematic review and meta-analysis (12). In some population surveys, the infection is a predisposing factor for IgE sensitization and asthma (13–19), while in others is protective (20–23). Recently, we discovered in the somatic extract of *Ascaris suum* distinct IgE-binding components recognized by sera of patients with asthma, some of them cross-reactive with mite allergens (24). In this review, we analyse the potential impact of this cross-reactivity on the pathogenesis of

IgE sensitization and the serological diagnosis of ascariasis and allergy.

RECENT PROGRESS ON THE IMMUNE RESPONSES TO HELMINTHS

Contemporary thinking on human immune responses to parasites is that they result from a long co-evolutionary process (25,26). Although they have several common mechanisms, immune responses vary according to the type of parasite (protozoa, helminths, species of helminth, etc.) and the genetic background of the host. One important feature of helminths is that they particularly induce a Th2 polarization that may be protective and also several regulatory mechanisms that could explain the parasitic relationship with the host. Epidemiological and experimental studies in humans suggest that the relative role of these components is not always the same. In a given population, a proportion of infected individuals are resistant to reinfections, while others are heavily parasitized. There are reasons to believe that this is strongly influenced by genetic factors in both host and parasite (1,25,27), and recent advances in elucidating the early cellular mechanisms induced by helminths infections will improve our understanding of the overall outcome.

It is widely accepted that intestinal parasites, such as nematodes, are controlled by a T-cell-dependent adaptive immune response where IL-4 and IL-13, as well as specific

antibodies, are important. The recent finding in mice that the protective response is associated with the early recruitment of previously unknown cells of innate immunity suggests the existence of an early type of Th2 response, non-T-cell mediated, but linked to it and induced by several cytokines from epithelial cells and other sources. For example, Moro *et al.* (28) found a cell population ('natural helper cells') in the mesenteric nodes of *Nippostrongylus brasiliensis*-infected mice that, in response to IL-33, produce large amounts of IL-13 and confer protection against this nematode. Neill *et al.* described 'nuocytes' as a group of cells that expand in mice lymph nodes under the influence of IL-25 and IL-33. Nuocytes, described as a 'new innate type 2 effector leukocyte', are an important early source of IL-13 during infection with the nematode *N. brasiliensis* (29). In addition, Saenz *et al.* identified the 'multipotent progenitor type-2 cells' that also increase in number when stimulated with IL-25. These are able to further develop into mast cells, basophils and antigen-presenting cells and, when transferred to IL-25 knock-out mice, provide enough IL-4, IL-5 and IL-13 to elicit protective immunity to infection with the nematode *Trichuris muris* (30).

Although the possibility that these cell populations share more than functional properties should be considered, they have in common the participation of IL-4 or IL-13 as important mediators of protective immunity to intestinal nematode infections. Interestingly, in addition to previous work on goblet cells' function in protection to parasites, another mechanism of action of these cytokines during infection with *Heligmosonoides polygirus* has been identified. This nematode induces intestinal epithelial cells to differentiate into goblet cells that secrete resistin-like molecule beta, which inhibits the ability of worms to feed on host tissues during infection, decreasing parasite adenosine triphosphate content and fecundity (31,32). Whether this mechanism of goblet cell differentiation also plays a role in the mucus production observed in experimental models of mite induced asthma (33) remains to be determined; however, it is worth mentioning the potential relationship of all these 'early type-2 innate immunity' expressions with the allergic response, especially where helminth infections are very frequent. We think that early recruitment of these types of cells supports the idea that co-exposure to intestinal nematodes and inhaled mite allergens during primary or secondary immune responses may result in boosting the allergic sensitization process.

During recent years, there has also been dramatic progress regarding the role of basophils in immunity to helminths, an aspect well documented in mice (34,35). Different animal models of infection show that helminths induce basophil proliferation, their migration to infected

tissues and release of cytokines such as IL-4 and IL-3, and chemokines that elicit a protective response of the immune system and epithelial cells. In the absence of IL-4- and IL-13-producing T cells, infection with *N. brasiliensis* is controlled by basophils, which seem to be sufficient to induce a primary protective immune response against the parasite (36). In infection models evaluating the secondary immune response, it has been observed that even without mast cells and T lymphocytes, basophils are able to elicit immunity against the parasite (37). Therefore, in addition to producing IL-4 and other conditions for polarizing Th2 responses after parasite infection or allergen exposure (38–40), basophils play a direct role in protecting against nematode infections in mice.

Extending this concept, Wada *et al.* (41) have demonstrated that these cells are also essential for the antibody-mediated acquired immunity against *Haemaphysalis longicornis* ticks in mice. However, the importance of dendritic cells (DCs) in Th2 immunity to parasites has also been confirmed (42), suggesting that the relative role of these two cell populations depends on the type of parasite infection. Moreover, Hammad *et al.* (43) have shown that inflammatory dendritic cells are necessary and sufficient for the induction of Th2 immunity to inhaled house dust mite allergen and propose that DCs initiate, and basophils amplify, Th2 immunity to this allergen source. This adds more elements to the complex scenario where immunity to helminths develops suggesting additional common pathways during parasite infections and the early immune response to environmental allergens.

In addition, it is important to point out that although immune mechanisms of defence against helminths in mouse models seem very effective (albeit variable in efficacy between strains of mouse), in humans the development of immunity to these infections is less evident. Even considering genetic influences, the obvious interpretation of the epidemiological data or the high frequency of reinfections (especially in children) among exposed communities is that immunity to helminths develops slowly in humans (25). The effects of this, often prolonged, host-parasite relationship on the inception and pathogenesis of atopy and allergic diseases will operate within the context of a strong immune response expelling parasites or strong suppressor mechanisms that inhibit appropriate immune effectors (Figure 2).

MODULATING EFFECTS OF PARASITE INFECTIONS ON THE HOST IMMUNE RESPONSE

The regulatory network associated with helminth infections has been extensively analysed (44–46). Some parasite

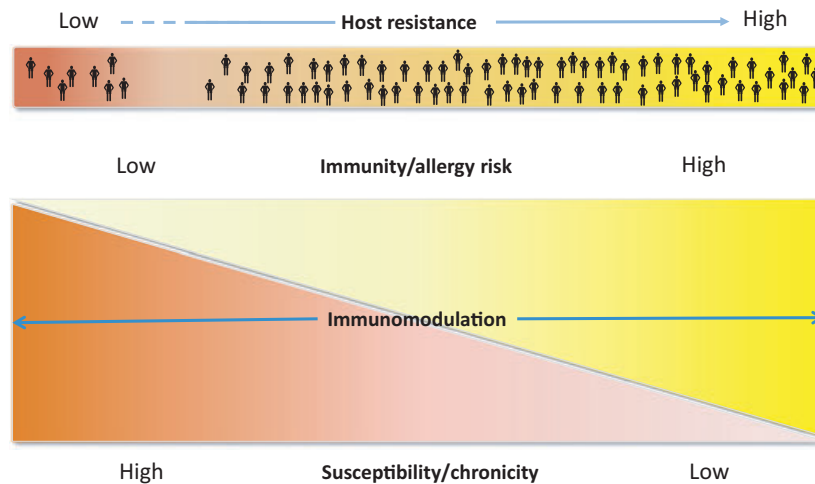


Figure 2 A hypothetical view of the relationship between host and *A. lumbricoides* during infection. Triangles represent gradients. Several points can be analysed: (a) *A. lumbricoides* infection is typically overdispersed. In a given population, genetic background will determine a minority that is currently susceptible to be highly parasitized. If asthma or atopy prevalence is looked at in this group, the low figures found may be attributable to this genetically determined hyporesponsiveness but also could be interpreted as a primary result of parasite-induced immunosuppression. The fact that there is evidence that immunity to *Ascaris* (as measured by IgE antibodies to the extract) is more frequent in populations with a high prevalence of allergic diseases makes more probably the first option. (b) The immunomodulatory effects of helminth infections are always present, in any degree, even in highly immune subjects. (c) Cross-sectional studies will provide relevant information if performed in the extremes of the gradients but may underestimate the real impact on the majority of the population. This model can apply for other helminth infections.

products prevent strong effector responses in the host, allowing the survival and reproduction of the parasite (47,48). It has been suggested that this may also affect the responses to allergens, leading to a lower prevalence of allergic sensitization in subjects that are chronically infected with high burdens of worms (44,49). Some mechanisms have been described using animal models (50,51) which include innate recognition, antigen presentation, T- and B-cell differentiation and antibody production. *Ascaris* contains lipids that stimulate Toll-like receptor 2 and induces the development of T regulatory cells (52) and phosphorylcholine-containing glycosphingolipids that significantly reduced proliferation of splenic B cells and inhibit IL-12 p40 production by peritoneal macrophages (53). Immunosuppressive cytokines also play their role (51); when using *A. suum* extract, this effect is primarily related to a downregulation of the antigen-presenting capacity of dendritic cells via an IL-10-mediated mechanism (54). The same may occur in humans because African children under hyper-endemic exposure to *A. lumbricoides* and *Trichuris trichiura* secrete more IL-10 and transforming growth factor β 1 than those under mesoendemic exposure (55). Some products of *Ascaris* downregulate the allergic response to bystander antigens like ovalbumin (56,57) when co-administered during the induction phase of allergen sensitization, but not during the effector response. IL-10-independent mechanisms also participate because the

pseudocoelomic fluid of *A. suum* inhibits the immune response to ragweed in an IL-10-deficient mouse (58). In addition, the suppressor effects of regulatory B cells during different types of experimental helminth infections, and their influence on allergic responses of mice, have also been described, and varying dependence on IL-10 has been detected (59–62). Although the role of helminth-elicited ‘alternative activated macrophages’ in immune downregulation is not clearly defined, this mechanism could be another way for maintaining the balance between immunity and tolerance or anergy in these infections (46).

The possibility that similar downregulatory processes occur in humans has been suggested by epidemiological surveys, most of them performed in rural populations suffering from chronic heavy worm infections (44,49,55,63). Interestingly, in those conditions, or in experimental animal models, the phenomenon may be accompanied by strong worm-specific Th2 responses (46) and does not severely affect IL-4 production or antibody synthesis (46,60,63). In addition, and will be considered later, there are experimental and epidemiological findings suggesting that *A. lumbricoides*-induced Th2 responses can promote allergic sensitization to other molecules in susceptible animals.

The realization that immunosuppression is associated with severe helminth infections in humans is very important for several reasons: first, it is another striking fact calling for the urgent eradication of parasitic diseases;

second, it has stimulated the search for new, parasite-derived immunomodulatory substances; third, it has improved our understanding of the immune system and parasitic relationships; fourth, it has provoked more questions, such as, to what extent does it impact the global prevalence of allergic diseases? This is an interesting point because it relates to more general issues such as the actual prevalence of allergic diseases around the world and their regional particularities, a problem that some researchers analyse within the framework of the hygiene hypothesis (64). In global terms, there are few reasons to believe that asthma and allergic diseases are less frequent in zones where parasitic diseases have not been eradicated. This probably occurs in some rural areas, but in underdeveloped countries, allergic diseases are very common in urbanized areas (65) and asthma and rhinitis are as frequent as in industrialized countries (66–75). The specific environmental risk factors leading to the remarkable differences in allergy prevalence between rural and urban communities remain unclear (76–78). The hypothesis that the immunomodulatory effects of parasite infections in rural settings explains it should be properly investigated.

CAN HELMINTH INFECTIONS INCREASE THE ALLERGIC RESPONSE?

In addition to the downregulation of allergic responses detected during some nematode infections (more evident and better studied in schistosomiasis than in ascariasis (79)), a strong IgE response dominates in human infections by *A. lumbricoides*, a phenotype that, for a long time, has been interpreted as potentially pro-allergenic and probably related to the complex lifecycle and the antigenic composition of this nematode. Also, high total IgE levels are typical of helminthiasis, which seems to be result of polyclonal B-cell stimulation by parasite products (80,81). The role of such nonspecific antibodies in immunity to parasites is unknown. Some authors have found that they may prevent cell sensitization by specific IgE (82), but there is evidence that a polyclonal IgE response does not prevent allergic reactions mediated by an actively produced IgE antibody (83,84). Therefore, other mechanisms, probably the immunomodulation on the effector phase of response, are currently considered when analysing the associations of helminth infections and skin tests with environmental allergens.

After penetration of the intestinal mucosa, *A. lumbricoides* larvae migrate to the liver, inducing the formation of granulomas, extensive inflammation and tissue injury. Surviving larvae reach the lungs and generate an inflammatory infiltrate in the airways dominated by severe peri-alveolar eosinophilia (85,86). Antibody production is

induced by larvae, and high levels of polyclonal and specific IgE are a hallmark of the infection and, in humans and pigs, immunity is determined by the generation of parasite-specific IgE antibodies against larvae and adult worms (87,88).

Experiments show that *Ascaris* induces sensitization and asthmatic symptoms in humans and infected animals, Loeffler's syndrome, and IgE-mediated asthma, including immediate-type cutaneous reactivity and airway responses after aerosol challenge with parasite extract (16,89–92). For example, Hagel *et al.* found that specific IgE levels to *A. lumbricoides* and positivity of skin test with the nematode extracts were associated with bronchial hyper-reactivity in children from a rural area of Venezuela. Also, the percentage of forced expiratory volume in 1-s (FEV1) predictive values correlated inversely with anti-*A. lumbricoides* IgE levels. In contrast, in urban children, the same associations were with specific IgE to *D. pteronyssinus* (16). As already mentioned, epidemiological investigations detected positive associations between *A. lumbricoides* infection and allergic phenotypes including mite sensitization (13–18).

It has therefore been proposed that the systemic enhancement of the Th2 response during helminth infection induces an allergic polarization to bystander antigens, such as aeroallergens (93,94). There is some experimental evidence supporting this contention. Earlier studies described that antigens of *A. suum* potentiate 'reaginic' response to ovalbumin (95,96). Also, *Ascaris* pseudocoelomic body fluid and the purified allergen ABA-1 prolonged the response to ovalbumin as third-party allergen, but they did not enhance the IgE levels to this allergen (97). In another investigation, co-administration of hen egg lysozyme with the excretory/secretory products of *N. brasiliensis* results in the generation of egg-lysozyme-specific lymphocyte proliferation, IL-4 release and IgG₁ antibody responses, supporting the role of some nematode products as adjuvants for third-party antigens (98). Furthermore, it has been shown that unidentified components in the body fluid of *Ascaris* promote a Th2 response and are adjuvants for specific IgE synthesis to some parasitic allergens like ABA-1 (57). Because, in addition to this allergen, *A. lumbricoides* extract has at least 11 human-IgE-binding components, the adjuvant effect may be more generalized (24), and because of co-exposure, this could happen for cross-reactive and non-cross-reactive mite allergens, a process that may have roots in the co-evolutionary relationship between worms and vertebrates (99).

Based on their findings from early epidemiological studies, Lynch *et al.* (100,101) suggested that the prevalence of allergies may be lower in individuals with high parasite burdens of geohelminths compared with those with low

burdens. This idea is now widely accepted and has been related to the acute and chronic clinical phenotypes observed in helminth-infected humans (102). In addition, intermittent mass de-worming programmes in preschool and school-aged children (103) reduce parasite burdens and boost the immune response to the parasites, because reinfections may elicit immune responses different in nature from the original primary infections (102). Therefore, it is theoretically possible that, in the presence of intermittent infections with low worm burdens, exposure to *A. lumbricoides* promotes allergic sensitization and asthmatic symptoms by increasing the synthesis of parasite-specific, mite-specific and mite-parasite cross-reacting IgE antibodies. The clinical impact may be particularly important in urban zones of underdeveloped countries, because in rural areas, the infections are usually more intense and associated with higher degrees of immunosuppression. Also, differences in mite fauna and levels of mite allergen exposure may influence the type of sensitization and, in consequence, the relevance of cross-reactivity.

IMMUNOLOGICAL CROSS-REACTIVITY BETWEEN DOMESTIC MITES AND ASCARIS

Cross-reactivity is a frequent feature of the adaptive immune response, involving antibodies or T lymphocyte receptors directed to diverse molecules (antigens or allergens) and resulting in diverse biological or clinical effects. It occurs when antibodies elicited to one epitope also recognize similar epitopes in other molecules. The allergen that is supposed to induce the original allergic responses is named the primary sensitizer, and the others are considered cross-reactive allergens. There are several clinical and laboratory criteria to classify an allergic reaction as cross-reacting, but the condition should be first empirically demonstrated (104).

The clinical relevance of IgE cross-reactivity has been described for foods, pollens, mites and other allergen sources (105), but its occurrence between mite and *Ascaris* allergens, although widely suspected (106), has not been thoroughly investigated. Cross-reactivity depends on amino acid sequences and conformational structures of the molecules, which explains why it is more frequent (but not exclusive) among phylogenetically related species. *Ascaris* and mites are related invertebrates and are expected to share several allergens. Independently of which source is the primary sensitizer, among inhabitants of the tropics, allergenic stimulus derived from a persistent inhalation of high concentrations of mite allergens and infections with *A. lumbricoides* may generate a particular immune response that involves cross-reactivity in both directions.

Several antigens of *Ascaris* have been analysed (50,107,108) and other are under scrutiny, but our knowledge about the allergenic composition of the whole extract is still very limited; in fact, the International Union of Immunology Societies only reports the ABA-1 allergen (Asc s 1) and the recently submitted tropomyosin (Asc 1 3). Because almost all allergens from domestic mites have been identified, it is now possible to study their cross-reactivity with *Ascaris*. We performed dose-response ELISA and immunoblotting inhibition studies with extracts of *B. tropicalis*, *D. pteronyssinus* and *A. suum*, demonstrating that there is a high degree of cross-reactivity between these sources including protein IgE epitopes (24). Although carbohydrate epitopes can be involved (109), inhibition of IgE binding was also demonstrated using deglycosylated extracts and nonglycosylated recombinant allergens. Using sera from patients with asthma, our experiments strongly suggest that mites are the primary sensitizers and that clinically relevant allergens such as tropomyosin and glutathione transferases are involved. Although, as suggested, the clinical relevance of cross-reactivity between parasites and house dust mites in tropical regions needs to be demonstrated (109,110), we postulate that the high prevalence of IgE antibodies to mites observed in tropical populations is partially the result of cross-reactivity with *Ascaris* allergens. Also, the high prevalence of allergy observed in urban areas of the tropics, even in places with poor hygienic conditions, may be influenced by the same phenomenon.

The potentially predisposing effect of IgE cross-reactivity on allergy inception and evolution may be also determined by the genetic background of the host (1,111–115). As mentioned, during infection, some subjects develop a strong Th2 response, possibly useful to eliminate the parasite (116) but adverse to the regulation of the immune response to other environmental antigens (117). The major histocompatibility complex can restrict the type of epitope recognized, making some individuals able to present nematode-specific antigens (such as ABA-1), while others present epitopes from cross-reactive allergens e.g. tropomyosin (118,119). Thus, it is possible that susceptible individuals become sensitized and develop symptoms after contact with cross-reacting allergens. Further studies are necessary to evaluate at the population level whether the IgE responses to nematode tropomyosins are more directed to cross-reactive epitopes or species-specific epitopes and whether patients with asthma have a particular predisposition to recognize cross-reactive epitopes. Recent genetic epidemiology studies in our laboratory have shown that genes controlling the IgE responses to *Ascaris* extract and ABA-1 may be different to those influencing specific IgE to mites (111). Thus, in addition to the duration and

degree of exposure, individual genetic susceptibility will have a role in determining whether subjects co-exposed to *Ascaris* and mite allergens become IgE-sensitized to nematode-specific antigens, mite-specific allergens or both.

ASCARIS LUMBRICOIDES TROPOMYOSIN (ASC L 3) AND THE ALLERGIC RESPONSE TO MITES

Tropomyosin belongs to a family of phylogenetically conserved proteins of eukaryotes and is considered to be an invertebrate pan allergen (120,121). Although most amino acids are conserved, some segments of sequence differ enough between vertebrates and invertebrates to induce IgE antibody responses in mammals (122). It is the major shrimp allergen (123,124) and also important in other species of crustaceans, molluscs and cephalopods (125,126). Also, it is a potent inhaled allergen from cockroach and mites and a recognized target for IgE antibodies during infection with nematodes (127–129). Mite tropomyosins are in group 10 allergens, e.g. Der p 10, Der f 10, Blo t 10, Lep d 10, Tyr p 10 (130–133). In crustaceans and molluscs, they belong to group 1, group 7 in cockroach (134,135) and group 3 in nematodes (Ani s 3 and Asc l 3).

Cross-reactivity between tropomyosins of crustaceans and mites has been reported (136–140) and, to a lesser extent, for mites and nematodes (141–143). Santos *et al.* (129) cloned a tropomyosin from *A. lumbricoides* and described a strong correlation between IgE levels to *Ascaris* and cockroach tropomyosins, although cross-reactivity was not experimentally evaluated. We recently demonstrated, by cross-inhibition ELISA, immunoblotting and mass spectrometry analysis, a very high allergenic cross-reactivity between the *B. tropicalis* tropomyosin Blo t 10 and the natural *Ascaris* tropomyosin using sera from patients with asthma (24). These results were confirmed using a recombinant *A. lumbricoides* tropomyosin expressed in a bacterial system (144), supporting the correlation between IgE levels to Blo t 10 and *Ascaris* tropomyosin, further implicating co-sensitization, resulting from a high degree of cross-reactivity. Amino acid sequence identity between these tropomyosins ranges from 73% to 74%, and some regions predicted as IgE-binding epitopes in shrimp tropomyosin were found to be identical in these molecules.

We also found that IgE antibodies to rAsc l 3 represent a high proportion (~50%) of the total IgE response to an unfractionated parasite extract, and there was allergenic equivalence between rAsc l 3 and the native counterpart in the *A. lumbricoides* extract. Moreover, the anti-tropomyosin IgE antibodies of sensitized subjects reacted against *A. lumbricoides* tropomyosin and induced mediator

release in effector cells, both *in vivo* and *in vitro*. The clinical impact of these findings relies on the particular environmental conditions of the tropics (especially urbanized areas of low income), where perennial exposure to high concentrations of mite allergens and intermittent infections with *A. lumbricoides* are common. In this setting, allergenic stimulation by cross-reacting tropomyosins may provide signals for sustaining IgE synthesis and perpetuate the allergic inflammation. Supporting this hypothesis, our mite-allergic patients with asthma are more frequently and more strongly sensitized to rAsc l 3 than controls, both groups being sensitized to the *Ascaris* extract (Figure 3). Because the main risk factor for asthma in the tropics is specific IgE to mites, it is possible that this pattern of reactivity is attributable to the exposure to cross-reactive tropomyosins (144). This mechanism may also explain, at a population level, why in tropical environments from Africa and South America, tropomyosins from mite and other invertebrates (e.g. cockroaches) constitute very important allergens, with sensitization frequencies above 50% (145,146), while in developed regions among mite-sensitized patients, tropomyosin is a minor allergen (5–16%) (130,147,148), probably because of the low concentrations of this allergen in the mite body.

These findings suggest that Asc l 3 influences the patterns of IgE responses to mite tropomyosins and may not be restricted to this allergen because *Ascaris* extract has at

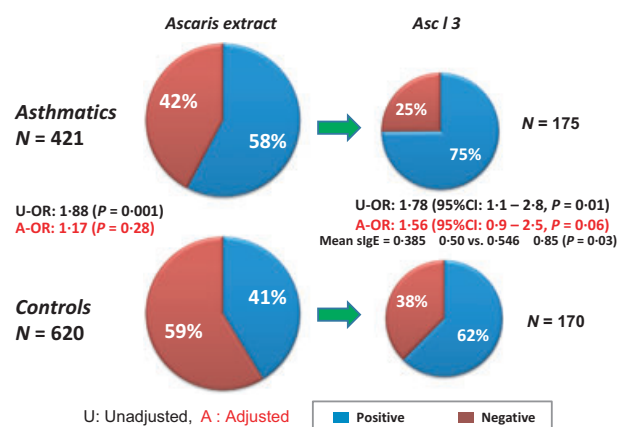


Figure 3 Distribution of IgE responses to *Ascaris* extract and tropomyosin (rAsc l 3). Mite-allergic asthmatic patients are more frequently and more strongly sensitized to rAsc l 3 than controls, both groups being sensitized to the *Ascaris* extract. The study population was from Cartagena, a tropical city in Colombia. All subjects lived in an urban, nonindustrialized setting and belonged to the lower 3 (of 6) socioeconomic strata in the city. The clinical characteristics of patients and controls have been described previously (111). Controls were recruited from the same neighbourhoods as the patients. Odds ratios (OR) were adjusted for specific IgE to *D. pteronyssinus* (144).

least 7 IgE cross-reactive components (200, 116, 77, 58, 40, 33 and 23 kDa) that may exert similar enhancer effects (24).

THE IMPACT OF CROSS-REACTIVITY ON THE SERODIAGNOSIS OF ASCARIASIS AND MITE ALLERGY

Conventionally, the diagnosis of *Ascaris* infection is achieved by the identification of parasite eggs in stool samples. However, the evaluation of *A. suum* infection in pigs shows that egg counts in faeces greatly underestimate the proportion of exposed individuals compared with anti-*Ascaris* IgG titration by ELISA (149). Similar findings were obtained in humans, where serodiagnosis of ascariasis, as detected by *Ascaris*-positive IgE, is three times the positive egg prevalence (150,151). It is well recognized that egg counting leads to a significant number of false negatives, and available immunoassays could be more sensitive (152). In addition, as specific IgE antibodies to helminths persist for a long time (153), serology allows the identification of previous contacts with *Ascaris*, even in egg-negative adolescents and adults; yet, this diagnostic tool also has the potential problem of the lack of specificity because of cross-reactivity.

In the search for useful serological markers to diagnose ascariasis, various antigen sources have been tested (154). Some have evaluated whole nematode extracts and others the pseudocoelomic fluid or preparations of excretory/secretory antigens. Currently, different reagents are under investigation including recombinant or purified antigens such as one of 24 kDa (155) and a specific somatic antigen of 34 kDa from adult *A. lumbricoides* (156). Because now it is clear that a high degree of cross-reactivity exists between *Ascaris* and mite extracts (24), this has to be added to the recognized problem of cross-reactivity between some proteins of *Ascaris* and other nematodes (156–159) and should be taken into account when assessing ascariasis using specific IgE or IgG against whole *Ascaris* extracts. In this circumstance, it is also necessary to start using component-resolved diagnosis, what means further basic research to isolate useful diagnostic components from *Ascaris* and mites. One important step has been achieved by M. Kennedy *et al.* who identified and cloned the abundant *Ascaris* allergen called ABA-1 (160).

ABA-1 (Asc s 1) is a member of the nematode polyprotein allergen/antigens (161–163). Studies support that immune responses (IgG and IgE) to ABA-1 are associated with previous infection and immunity to *Ascaris* (152). In endemic regions, the antibodies isotypes to ABA-1 correlate with the severity of infection, being IgE associated

with low infection levels and IgG₄ or seronegativity with higher susceptibility to the infection (88). This protein of 15 kDa has only been found in nematodes, has fatty acid-binding properties (164) and is synthesized as a polyprotein in gut of the worms and released into the pseudocoelomic fluid of the parasite (161,165). We found no cross-reactivity between ABA-1 and any component of the *D. pteronyssinus* and *B. tropicalis* extracts, confirming its usefulness as a more specific marker of *Ascaris* infection, avoiding the bias of cross-reacting mite allergens. However, the sensitivity and specificity of tests with ABA-1 should be further evaluated because homologous molecules like gp15/400 ladder protein of *Brugia malayi* (166) and TBA-1 from *Toxocara* ssp. (167) may affect the utility of the assay.

Another aspect of this problem is the impact of cross-reactivity in the diagnosis of mite allergy. It is generally accepted that total IgE is not a good diagnostic parameter for allergy in the tropics because parasite infections increase serum levels of this immunoglobulin (168). For that reason, the quantification of specific IgE to mite extracts is considered a mandatory alternative. However, *Ascaris* cross-reactive allergens may influence mite allergy diagnosis when using the whole mite extracts, as is routinely done *in vitro* and for skin testing. Therefore, in the tropics, the use of complete mite extracts for diagnosis could lead to false positive results. Also, the potential complications of immunotherapy with mite extracts under the influence of cross-reacting antibodies to *Ascaris* components deserve more investigations.

Cross-reactivity between mite and *Ascaris* should also be considered when interpreting surveys analysing the role of ascariasis as a risk factor for allergies. Most of these studies have measured the levels of specific IgE to *Ascaris* extract as a marker of exposure, comparing it between allergic patients and controls and obtaining variable, often contradictory results. The influence of cross-reactivity could be exerted through the high frequency of IgE sensitization to mites among cases, especially in patients with asthma; in some studies, sensitization to *Ascaris* may be apparently associated with asthma because of cross-reacting antibodies. Although statistical methods are helpful to analyse the relative weight of these effects, the definition of which proportion of antibodies to *Ascaris* extract actually cross-react with mite allergens and their relative effect in conferring risk can only be obtained experimentally in animals, and in humans using component resolved diagnosis. Some studies have performed such statistical analyses; interestingly, when mite sensitization is included as covariate, some associations remained and other disappeared. For example, in Costa Rica, specific IgE to *A. lumbricoides* extract was a risk factor for the

number of positive skin test or bronchial hyper-reactivity; however, the significance disappeared when adjusting for specific IgE to mites and cockroach (15). Of course, this does not rule out a biological effect of *Ascaris*-specific antibodies on the phenotypes; instead, it supports the potential pathogenic effects of both mite and *Ascaris* sensitization. Indeed, in another study, *Ascaris*-specific IgE was an independent risk factor for wheezing even when adjusting for anti-mite antibodies (14). Therefore, the relative effect of cross-reactivity will vary depending on the level of exposure to *Ascaris* or mites, the primary sensitizer, housing styles and type of environment (urban or rural). Unfortunately, most studies do not evaluate mite fauna or mite sensitization in the population, making even more difficult the interpretation of results.

HOW TO INVESTIGATE A BOOSTING EFFECT OF ASCARIASIS ON ALLERGIC SENSITIZATION?

In this review, we hypothesize that, because of cross-reactive molecules; mild intermittent urban infections with *A. lumbricoides* potentiate the IgE response to mite allergens and, in consequence, influence the evolution of mite sensitization and asthma. This has to be properly evaluated using the necessary approaches and tools. One important analysis would be the assessment of *A. lumbricoides* infection or sensitization to this nematode as risk factors for allergy, and a number of studies have addressed this point, some of them detecting positive associations and others the opposite. However, the contradictory results of these cross-sectional surveys performed with different methodologies in different populations and at different times in the course of the infections are not unexpected. Other strategies, epidemiological and experimental, should be used to investigate the problem, as are currently being used by some groups. For example, the inclusion of more specific serologic markers for *Ascaris* and mites will improve the accuracy of current and future epidemiological studies. Also, the follow-up of the IgE immune

responses and allergy symptoms in birth cohorts of children exposed to mites and parasites will help to elucidate primary sensitizers and analyse the interactions between atopy and immunity to helminths. At the experimental level, animal sensitization with mite allergens during infection with nematodes may address the question of boosting effects more directly.

CONCLUDING REMARKS

In many tropical countries, the environmental conditions make possible co-exposure to domestic mite allergens and nematodes like *A. lumbricoides*. A high degree of IgE cross-reactivity between these sources has been demonstrated, but its effects on the inception, evolution, diagnosis and therapy of allergic diseases are unknown. We hypothesize that perennial immunological boosting from invertebrate cross-reactive allergens enhances allergic sensitization and sustains high levels of specific IgE. In this way, cross-reactivity contributes to the complex interactions that determine the pathogenesis of allergic diseases in the tropics and explains the high prevalence of IgE sensitization to invertebrate allergens as well as the high frequency of asthma and other allergic diseases detected in the urban settings where epidemiological studies have been performed. According to the hygiene hypothesis, it is expected that the high microbial exposure owing to poor hygiene conditions in underdeveloped countries leads to low prevalence of allergic diseases. Helminth infections may explain why a number of epidemiological surveys have found the contrary.

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