Cross-Reactive IgE Responses to Invertebrates

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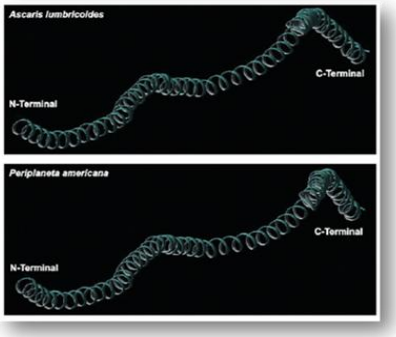
Introduction

In clinical practice, we often find patients who present positive skin tests and/or detectable IgE antibodies to ntigens derived from invertebrates, including shrimp, mites and cockroach. One issue that arises it whether these IgE antibody responses are truly specific or whether they might represent cross-reactive IgE responses to shared allergens, which may or may not be clinically relevant.

One example is the case of sensitization to shrimp and other crustacea and mollusks. Once patients are given a diagnosis of shellfish allergy, they typically avoid all shellfish for life. Therefore it is important to provide the patient with a precise diagnosis of shrimp allergy, in order to avoid unnecessary restriction diets. However, current methods for *in vivo* and *in vitro* diagnosis of shrimp allergy show low specificity relative to outcomes of double-blind placebo-controlled challenges with shrimp. A recent study by Ayuso et al[1] revealed that children with shrimp allergen recognize a greater number of epitopes, with higher intensity, as compared to adults, when evaluated by microarrays with peptides from the four allergens identified in shrimp: tropomyosin, arginine kinase (AK), myosin light chain (MLC) and sarcoplasmic Ca-binding protein (SCP). Interestingly, the results confirmed that tropomyosin is the major shrimp allergen, both for children and adults, and suggested that IgE reactivity to shrimp allergens may decrease over time[1].

We have focused our studies on the role of tropomyosin in IgE responses to cockroach and *Ascaris lumbricoides*. Tropomyosins are highly conserved proteins that have been shown to be pan-allergens in invertebrates including shrimp and other crustaceans and mollusks, mites, and cockroach. In addition, tropomyosin has been identified in *Anisakis simplex*, a fish parasite that can cause allergic reactions in human beings. The high degree of amino acid sequence identity among invertebrate tropomyosins provides support for immunological cross-reactivity, and some studies have suggested that this cross-reactivity may be clinically relevant. For example, IgE antibody reactivity to shrimp among Orthodox Jews, who are prohibited from eating shellfish, is thought to be a result of tropomyosin.

Sequence identity of *A. lumbricoides* tropomyosin and tropomyosins of invertebrates



Tropomyosin origin	% Identity
Anisakis simplex	98%
Onchocerca volvulus	95%
Dermatophagoides farinae	74%
Dermatophagoides pteronyssinus	73%
Blattella germanica	69%
Periplaneta americana	69%
Lobster	74%
Shrimp	71%
Schistosoma mansoni	57%

Infections with parasites and allergic diseases

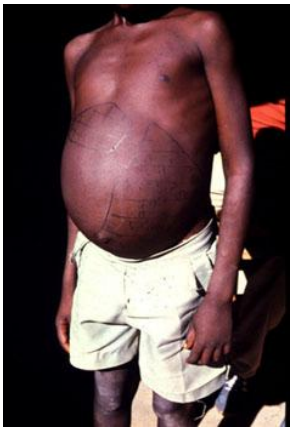
Epidemiological and experimental studies support a link between infections with helminth parasites and reduced incidence of allergic diseases. Recent data from clinical trials, involving intentional infection with parasitic helminth larvae (*Necator americanus*) or treatment with helminth ova (*Trichuris suis* ova, TSO) have been inconsistent in showing significant therapeutic benefits in patients with asthma and allergic rhinitis. Worm therapy may be a new potential approach to improve allergic conditions, however many aspects including mechanisms, practical regimens and safety need to be evaluated.

Helminth infections cause significant morbidity in endemic countries, leading to malnutrition, anemia, delays in development and impairment of cognitive functions. Regular deworming programs for a number of these diseases, particularly addressing schistosomiasis and infections by soil transmitted helminths, are currently strongly recommended by the WHO, to be implemented as a public health priority. Helminth infections have strong modulatory effects on anti-parasite inflammatory responses in the human host, which also play a role in limiting vaccine efficacy interfering with immunity to heterologous infections. These observations clearly indicate that efforts to use antihelmintic treatments are justified to maximize protection against the major childhood microbial diseases. Therapy for allergic diseases with worms would be unacceptable and unethical in most situations. However, it would be of great interest to define and characterize specific helminth molecules associated with consistent and strong immunomodulatory effects as targets for application in the treatment or prophylaxis of allergic conditions.

The issue of whether infections with parasites promote or protect from development of atopy and asthma remains controversial. Several studies have demonstrated a negative association between helminth infections and allergy, however some studies point to the opposite direction. The discrepancy has been tentatively explained by differences in frequency, dose, time, and type of helminth.[2] There is consisted evidence that infection with schistosoma induces a strong regulatory network which includes activation of regulatory T-cells and production of high levels of IL-10, both in experimental setting and among patients from endemic areas, leading to protection from atopy and attenuation of symptoms of asthma. Likewise, there is data showing that hookworm infection may protect against asthma. On the other hand, there is evidence to indicate that infection with *Ascaris lumbricoides* is a risk factor for wheezing and asthma in different populations.[3]

The hypothesis that parasitic helminths may protect against the development of allergic and autoimmune conditions has led to the assessment of the protective effects of parasitic worms and their immunomodulatory products in model systems. Analysis of the mechanism of action of these immunomodulators has revealed various structurally distinct helminth products that promote activation of regulatory T-cells, or act by reprogramming TLR signalling from a pro- to an anti-inflammatory phenotype. One example is the schistosome derived lysophosphatidylserine, which can mediate subversion of TLR2 signalling towards an anti-inflammatory phenotype.[4]

Schistosoma antigens



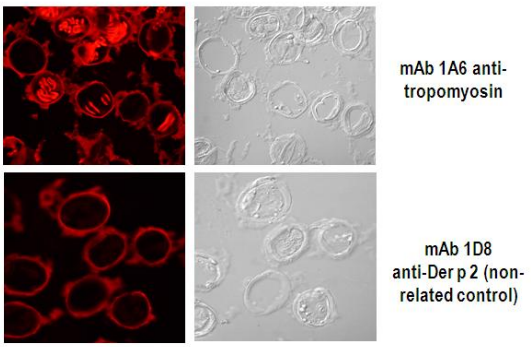
Several *S. mansoni* antigens have been tested as vaccines to prevent *S. mansoni* infection and to avoid liver pathology, including Sm22·6, Sm29 and PIII. The Sm22·6 antigen is a soluble protein associated with the tegument of *S. mansoni*, present throughout the life cycle of the parasite, with the exception of the egg stage. It has been shown that recombinant Sm22·.6 induces partial protection (34·5%) against experimental *S. mansoni* infection and also induces high levels of IL-10 production. Sm29 is a membrane-bound glycoprotein found on the tegument of the adult worm during the lung stage of *S. mansoni* infection. This protein induces a Th1 cytokine profile in mice and provides 50% protection against infection. PIII is a multivalent antigen obtained from the *S. mansoni* adult worm antigen (SWAP); it modulates granuloma size in mice infected with *S. mansoni*. Cardoso et al, working in Northeast Brazil, have shown that these three antigens were able to down-modulate allergic inflammatory responses in a murine model of airway inflammation and suggested that the CD4+FoxP3+ T cells might play an important role in this process.[5]

Schistosome-derived lysophosphatidylserine contains a helminth-specific acyl chain that interacts with Toll-like receptor 2 (TLR2) leading to differentiation of dendritic cells that induce IL-10 producing regulatory T cells. Similarly, lacto-N-fucopentaose III (LNFPIII)/Lex, which contains the Lewis X trisaccharide, can induce ex vivo production of IL-10 and prostaglandin E2 by B-1 B cells from schistosome-infected mice. Other examples of potential therapeutic components of helminth parasites include omega-1, a glycoprotein derived from schistosome eggs that specifically primes Dendritic Cells to drive polarized Th2 responses; and ES-62, a secreted phosphorylcholine-containing glycoprotein of the filarial nematode *Acanthocheilonema viteae,* which conditions DCs to induce Th2 responses via activation of TLR4. Interestingly, the two distinct helminth products, ES62 and LNFPIII, signal through TLR4, however unlike LPS, induce anti-inflammatory responses, whereas schistosome derived lysophosphatidylserine is an example of a helminth-derived antigen that can mediate subversion of TLR2 signaling towards an anti-inflammatory phenotype.[2,4]

Ascaris lumbricoides

Our group has reported for the first time the sequence, molecular cloning and recombinant expression of tropomyosin from *A. lumbricoides.* Tropomyosin, a muscle protein that is highly conserved throughout the animal kingdom, is present in all helminths. Invertebrate tropomyosins account for widespread allergic reactions against seafood, house dust mites and cockroach. Due to this crossreactivity, invertebrate tropomyosin is considered to be a panallergen. Interestingly, vertebrate tropomyosins are not allergenic.

Tropomyosins from parasites share a high degree of amino acid sequence identity, and induce IgE antibody responses. Interestingly, tropomyosin it is one of the most prominent candidates for an anti-nematode vaccine to treat filarial infections.[6]



Ascaris eggs containing L3 stage larvae

Our data revealed that *A. lumbricoides* tropomyosin is abundant both in the adult worm and in the L3 larvae, which is the stage of pulmonary passage of the parasite, and that it presents extensive immunologic cross-reactivity to cockroach tropomyosin. These observations have led us to formulate the hypothesis that exposure to *A lumbricoides* L3 larvae in the respiratory tissue early in life could enhance TH2 polarized responses. In endemic areas, children often get infected with Ascaris through ingestion of parasite eggs before the first year of life. Most of the larvae passing through the lung tissue die locally, allowing release of antigens including tropomyosin to be taken up by antigen-presenting cells, which in turn undergo migration to regional lymph nodes to stimulate TH2 responses and IgE production. Progressively, these children also get exposed through the inhalation route to allergens derived from mites and cockroach, which share the highly homologous tropomyosin. It is possible that IgE responses to tropomyosin derived from inhalant allergens could be amplified or develop more promptly as a result of previous sensitization to Ascaris tropomyosin, triggering persistent lung inflammation.[7] One interesting question would be whether immunotherapy with tropomyosin either by subcutaneous or sublingual route would induce protection both to allergen and to *Ascaris* infection.

Other allergens with potential cross-reactivity

The potential confounding effect of other cross-reactive arthropod allergens, such as AK and MLC, requires further evaluation. For example, shrimp AK has 82% sequence identity with the cockroach *Periplaneta americana* (Per a 9), and 78.7% identity with AK from the dust mite *Dermatophagoides pteronyssinus* (Der p 20). In addition, shrimp MLC has significant sequence identity (52%) with cockroach MLC (Bla g 8). This emphasizes the need for component-based diagnosis with recombinant shrimp allergens to evaluate subjects with shrimp allergy and subjects with shrimp tolerance but arthropod allergy[1].

Conclusions

Although there is consistent evidence that some parasite infections may protect against allergy and asthma, giving parasite infections to patients to treat or prevent allergic diseases is not an acceptable strategy due to associated risks. However, it would make sense to focus on parasite molecules which induce immunomodulatory effects. A few molecules have been identified that could make suitable candidates for therapy. Future studies will establish whether these molecules would be useful in the clinic, as a sole therapy, or possibly coupled to allergens to target allergen specific immunomodulatory responses.

References

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