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Parasite allergens

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ABSTRACT

Human IgE against helminths is a normal component of the whole protective response elicitesd during infection, when specific IgE to a great number of antigens is produced; however, few of those IgE binding components are actually allergens. In general, considering the strong Th2/IgE responses during helminth infections is intriguing that they are not usually associated with allergic symptoms, which probably (but not exclusively) depends on parasite-induced immunomodulation. However, allergic manifestations have been described during some helminth infections such as ascariasis, strongyloidiasis, anisakiasis and hydatidosis. In addition, there is evidence that helminthiases (e.g. ascariasis) can increase symptoms in allergic patients. Furthermore, allergic reactions during anti-helminth vaccination have been observed, a problem that also could be associated to the future use of parasite derived immunomodulators. Therefore, identification and characterization of helminth allergens is a matter of increasing research and a great number of IgE binding antigens have been found (www.allergen.org and www.allergome.org). Here we describe only a small group of them, for which allergenic activity (the ability to induce IgE mediated inflammation) have been clinical or experimentally demonstrated. *Ascaris lumbricoides* tropomyosin (Asc l 3) has strong allergenic activity; in the Tropics it has been associated with asthma and asthma severity, suggesting clinical relevance. In addition, due to its cross reactivity with mite tropomyosins this allergen could influence house dust mite (HDM) allergy diagnosis. Characterized *Ascaris* allergens also include the polyprotein As s 1 (ABA-1) and the Glutathione transferase As l 13. Other helminth allergens include *Anisakis simplex* Ani s 1, Ani s 4, Ani s 7 and Ani s 9; *Necator americanus* NaASP2q and Nacal1 and *Schistosoma mansoni* SmVAL4 and Sm22.6. Future work on helminth IgE binding antigens will help to understand several aspects of allergenicity and allergenic activity, among them the increasing finding of IgE binding molecules that not induce allergic symptoms.

1. Immunology and epidemiology

1.1. A. lumbricoides allergens

A. lumbricoides is the most common soil-transmitted nematode and compared to other helminth species is very allergenic. Several epidemiological surveys have found that the IgE response to this helminth is a risk factor for asthma and atopy and is stronger in mite-sensitized allergic patients. Three *A. lumbricoides* allergens are listed in the official WHO/IUIS site, one species specific (Asc s 1, also known as ABA-1) and two cross reacting (Asc l 3 and Asc l 13), although at least nine additional IgE binding components have been reported. Tropomyosin, a well-recognized pan-allergen, is involved in Ascaris-HDM cross-reactivity, which is explained by the high degree of structural homology between Asc l 3 and HDM tropomyosins (Acevedo et al., 2009; Acevedo et al., 2011). Specific IgE levels to Asc l 3 are significantly higher in asthmatic patients compared to healthy controls which suggest that it may be risk factor for asthma symptoms in the Tropics (Ahumada et al., 2015). It has been proposed that patients predisposed to asthma, with a strong pro-Th2 genetic background, early age parasited, suffering several re-infections and permanently exposed to mite allergens probably have a stronger IgE response to allergens and more severe clinical symptoms (Ahumada et al., 2015; Hagel et al., 2007; Lopez et al., 2002). The pseudocelomic fluid of Ascaris contains abundant amounts of the nematode polyprotein ABA-1 (Asc s 1). In humans, the IgE and IgG responses to ABA-1 has been associated with protection (McSharry et al., 1999) rather than allergy symptoms, and no association has been found between IgE sensitization to ABA-1 and asthma. Indeed, ABA-1 is a species specific antigen and is considered a nematode specific infection marker; it does not cross-react with any of the *Blomia tropicalis* or *Dermatophagoides pteronyssinus* allergens. The frequency of IgE reactivity to Asc l 13 is low (< 20%) but these molecules exists as isoforms and may be clinically relevant in cases where there is also sensitization to mite and cockroach GSTs (Acevedo et al., 2013). The

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allergenic cross reactivity between Ascaris and HDM GSTs is suggested by the structural homology between Asc l 13 and allergenic GSTs in cockroach (Bla g 5) and HDM (Der p 8 and Blo t 8); however, more studies are needed to confirm this. The additional discovery of allergenic properties of Asc l 13 may explain why the frequency of IgE sensitization to invertebrate GSTs is higher in the Tropics than in populations living in temperate areas (Weghofer et al., 2008).

1.2. Anisakis simplex allergens

A. simplex is the most allergenic helminth. In fact, its harmful effects do not depend on parasitism but on the allergic reaction they generate in the host. This is in contrast to other nematodes such as *A. lumbricoides*, which in spite of inducing large amounts of specific IgE antibodies against several of its components, the generation of clinically important allergic reactions during infection is uncommon. The reasons for these phenomena are unknown, but could be due to a low immunosuppressive activity of *Anisakis*. Officially designated Ani s 1 to Ani s 14, their allergenic activity, according to the criteria of the WHO/IUIS Committee, has been verified in only four of them: Ani s 1, Ani s 4, Ani s 9 and As 22U. Seven, including Ani s 1, Ani s 4, Ani s 5, Ani s 6, Ani s 7, Ani s 8, Ani s 9 are parasite excretion/secretion molecules and four, Ani s 1, Ani s 2, Ani s 3 and Ani s 12 are somatic antigens (Pravettoni et al., 2012).

Ani s 1 is a thermostable 24 kDa serine protease inhibitor with 86% IgE and 29% IgG binding among *Anisakis* allergic subjects. Ani s 1 is considered the main allergen of this species. Both the native and recombinant molecules are recognized by specific IgE and the recombinant generates positive skin tests at concentrations of 10 µg/ml (mean papule size 20.6 mm2), in 100% of subjects allergic to *Anisakis* and negative in all non-allergic controls. The recombinant also activates basophils in all subjects with positive SPS at Ani s 1 (Gamboa et al., 2012). Ani s 4, the *Anisakis* cystatin, is a thermostable protein produced in the secretory gland and the cuticle basal layer of the larval state L3. It is recognized by IgE of 27% of allergic subjects and seems to be central in the induction of anaphylaxis since, in sensitized individuals, it activates basophils at concentrations of 0.01–1 µg/ml, reaching 91.7%, whereas in those patients sensitized to the extract but not to Ani s 4 the activation is only 1.1%, similar to negative controls (Rodriguez-Mahillo et al., 2007). Ani s 4 has a natural isoform containing proline instead of leucine at the 3 position of the mature protein. A study compared the IgE binding and allergenic activity of the recombinant Ani s 4-P isoform and the recombinant Ani s 4-L using basophil activation test. The Ani s 4-P isoform had stronger IgE binding in the sensitized subjects. Inhibition experiments showed different patterns. Ani s 4-L could almost completely inhibit IgE binding, whereas Ani s 4-P inhibited a maximum of 50% and its rate of self-inhibition was 75%. There was greater basophil activation by the L form starting at 0.001 µg/ml, suggesting that it has more allergenic activity than the P isoform (Rodriguez-Mahillo et al., 2008).

Ani s 7 (139 kDa) is also a major allergen of *Anisakis*; the native form is glycosylated and recognized by IgE from 100% of subjects infected, which gives it a great diagnostic value (Anadón et al., 2009). Several structural features support its allergenicity; one is the repetition of 19 motifs in tandem, a pattern also observed in the allergens Bla g 1 and Per a 1, although less homogenous in Ani s 7. The second is its high content of cysteines (4 residues per repetition) where immunodominant IgE epitopes are located (Rodriguez et al., 2008); however, there is no experimental support of the allergenic activity of this molecule.

Ani s 8 and Ani s 9 are two proteins of 15 and 14 kDa recognized by 25 and 13% of IgE from subjects sensitized to *Anisakis*. Both belong to the SPX/RAL-2 family (Kobayashi et al., 2007). Ani s 9 has 60% sequence identity with *Ascaris suum* AS14 antigen and 41% with *Acanthocheilonema viteae* antigen RAL-2 (Rodriguez-Perez et al., 2008). The allergenic activity of Ani s 9 and Ani s 1 was recently tested in a murine model of airway inflammation, in which mice were immunized with a mixture of OVA (ovalbumin) and the recombinants Ani s 1 or Ani s 9. A bronchial Th2 inflammatory response to both allergens was found. Total and specific IgE increased significantly, as did the cytokines promoting the allergic response (Cho et al., 2014).

1.3. Necator americanus allergens

There are two important IgE binding components of *Necator americanus*: NaASP2 and NaCalreticulin. NaASP2 is a 21.3 kDa protein secreted by the L3 larval stage that facilitates the entry to the host. It shares structural homology with the PR1 family (proteins related to pathogenesis 1) within the superfamily of extracellular proteins SCP/Tpx-1/Ag5/PR-1/Sc7 (Osman et al., 2012). Vaccination of hamsters and dogs with AcASP2 reduced adult parasite burden, fecundity and migration of larvae to the tissue; in addition, people living in endemic areas and having high levels of IgE to NaASP2 have a lower risk of reinfection (Bethony et al., 2005). Clinical trials with *N. americanus* vaccine candidates are described below.

NaCalreticulin (Na-cal-1) is a 45.6 kDa protein with high identity with calreticulins from other helminths. This antigen binds IgE from all subjects with antibodies to *N. americanus* extract (Pritchard et al., 1999). Nacal1 binds to the C1q component of the human complement, which is considered an immune evasion mechanism. The recombinant protein was proposed as a vaccine since it induced protection to the infection in mice (Winter et al., 2005), but later its allergenic activity was detected studying a parasitized population in Papua, New Guinea. Nacal1 induced dose dependent degranulation of basophils and histamine release at concentrations from 0.01 to 10 ug/ml. Histamine release was also induced by a preparation of excretion-secretion products of the parasite, being significantly greater than the control performed with non-parasitized subjects from the United Kingdom. All subjects in the study had high total IgE, between 677 and 26,467 IU/ml vs. 12–308 IU/ml in non-parasitized controls (Pritchard et al., 2007). The results from the above studies show that these two molecules of *N. americanus* are allergenic. Given the natural propensity of helminth antigens to induce a Th2 response during infection, it should be necessary to determine the specific IgE levels in endemic areas as a critical step for selecting potential vaccines or immunomodulators.

1.4. Schistosoma mansoni allergens

The *S. mansoni* transcriptome detected additional genes and proteins expressed on the surface of the parasite. According to the functional classification by Gene Ontology, they interact directly with the host's immune system, including a group of proteins encoded by genes with orthologues in wasps. This family was formally named as Venom-Allergen-Like proteins of *Schistosoma mansoni* (SmVALs). Some SmVALs (group 1) are proteins secreted at different stages of the life cycle (Rofatto et al., 2012) and most of them are strong immunogens, also inducing human specific IgE. Some SmVALs have been proposed as candidates for vaccines or immunomodulators, but one of the main concerns is their potential allergenic properties (Chalmers et al., 2008); therefore, the interest for determining their allergenic potential has been increased in the last years.

The allergenic activity of SmVAL4 and SmVAL26 was evaluated in a murine model of allergic inflammation. SmVAL4 is a protein released during the transition between cercaria and schistosomula, while SmVAL26 is released by eggs. Both were produced as recombinants in eukaryotic expression system to keep their glycosylation. The recombinants were used to sensitize and challenge BALB/c mice, using a positive control with OVA and an untreated group. Passive cutaneous anaphylaxis (PCA) tests were also performed to evaluate IgE antibodies generated by immunizations with rSmVAL4, rSmVAL26 and rSmVAL4-pro (a control treated with pronase for deglycosylation). The mice sensitized and challenged with rSmVAL4 showed increase number of bronchoalveolar lavage cells, especially eosinophils, whereas those