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Parasite allergens

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ABSTRACT

Human IgE against helminths is a normal component of the whole protective response elicited 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 polypeptide 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 polypeptide 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 (Rodríguez-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 (Rodríguez-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 (Rodríguez 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 (Rodríguez-Pérez 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 µg/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

sensitized and challenged with rSmVAL26 showed only a slight increase in the number of total cells, mainly macrophages. In mice treated with SmVAL4 a dense peribronchial inflammation was observed, not seen in the control or SmVAL4-Pro (non-glycosylated). SmVAL4 induced a significantly high production of IgE and IgG1 in comparison to SmVAL26, which produced mainly IgG2a; in PCA SmVAL4 induced positive reaction (Paiva Farias et al., 2012).

Other types of IgE binding proteins of *S. mansoni* are those of the TAL family (Tegumental allergen-like), present in the parasite tegument with different expression profiles during the life cycle. The Sm22.6 protein (SmTAL1) belongs to this group, as well as Sj22.6 of *S. japonicum* and Sh22.6 of *S. haematobium*, being all dominant targets of human IgE during infection (Dunne et al., 1992; Santiago et al., 1998; Fitzsimmons et al., 2004). Collectively, these proteins are characterized by having two EF-hand motifs in the N-terminal region, a characteristic shared by several clinically important allergens (Radauer et al., 2008). There are two additional proteins in the adult stage of the parasite, SmTAL2 and SmTAL3. SmTAL2 is also present in the eggs. The levels of IgE to SmTAL1 and SmTAL3 are high in infected population from endemic regions, increasing when the parasites died after treatment, while IgE levels against SmTAL2 were low during the course of infection and did not increase with treatment (Fitzsimmons et al., 2007). The existence of 32 *Schistosoma* antigens that induce IgE production in humans has been reported in an infected population of 222 individuals in Southeastern Uganda. The dynamics of antibodies to several Allergen-like proteins before and after treatment with praziquantel showed that the most prevalent pattern of response during infection was high IgG4 and low IgE titers, although for some antigens high IgE response prevailed (Farnell et al., 2015).

Since SmTAL1 (Sm22.6) induces high levels of specific IgE associated with protection from reinfection, it has been proposed as a candidate for a vaccine against schistosomiasis. The potential allergenic activity of this molecule was explored using the method of functional evaluation of antigen-specific IgE based on the expression of the NFAT-dependent luciferase, which is induced by IgE-dependent crosslinking in the humanized cell line from rat basophilic leukemia (RS-ATL8). Sera from subjects infected with *S. mansoni*, who had specific IgE against SmTAL1 and SmTAL2 were used. A pool of sera from subjects allergic to *Parietaria judaica* with high levels of specific IgE against Par j 1 was used as control. The antigens were recombinants expressed in *E. coli* (rSmTAL1 and rSmTAL2) and also in wheat germ. The rSmTAL1 expressed in both systems activated basophils with significantly higher luminescence values compared to the controls and even higher with the recombinant expressed in wheat germ. rSmTAL2 expressed in *E. coli* did not activate basophils but that expressed in wheat germ did (Wan et al., 2014). Having allergenic activity is a limitation for helminth vaccine candidates.

2. Biology and biochemistry

Helminth allergens belong to well-known families of common allergens (Table 1) and have different biological functions. *A. lumbricoides* tropomyosin (Asc l 3) has similar functions than other tropomyosins, highly related actin binding proteins playing an important role in the contractile activity and the regulation of cell morphology and motility. Tropomyosin family has several genes and a great number of isoforms; however there are only 2 reported Asc l 3 isoforms. The polypeptide As s 1 (ABA-1) is a fatty acid binding protein that may be crucial for the parasite acquisition, protection and distribution of lipids (Kennedy et al., 1995; Meenan et al., 2011); this type of protein is only found in nematodes and belongs to the Nematode Polypeptides family. The recombinant molecule represents a single A-type repeat unit of the ABA-1 polypeptide (ABA-1A), with only a few amino acid differences with *A. lumbricoides* ABA-1, being both fully immunologically cross-reactive (Christie et al., 1993). Glutathione-S-transferases play a key role in detoxification of reactive oxygen species as well as xenobiotics, and

Table 1

Helminth IgE binding molecules and their location in allergen families.

Allfam 2016	Family or superfamily	Helminth molecules
AF007 AF146	EF-hand proteins Tegumental allergen-like Calcium-binding proteins with helix-loop-helix motifs	<i>Anisakis simplex</i> : Troponin C <i>Brugia malayi</i> : BmTAL1 <i>Schistosoma</i> spp: SmTAL1, SjTAL1, ShTAL1, SmTAL2, SmTAL3, SmTAL4, SmTAL5, SmTAL13 <i>Ascaris lumbricoides</i> : Asc l 3 <i>Brugia malayi</i> : Bm3 <i>Onchocerca volvulus</i> : OvTrp <i>Schistosoma</i> spp: SjPM, SmpPM <i>S. mansoni</i> : Sm14 <i>Echinococcus granulosus</i> : FABP1 <i>Schistosoma</i> spp: SmVAL4, SmVAL26, SmCa <i>Ascaris</i> spp: As16
AF054 AF100	Tropomyosins and paramyosins	<i>Wuchereria bancrofti</i> : Wb14 <i>Necator americanus</i> : Nec a ASP-2
AF045	Cupins	<i>Ancylostoma caninum</i> : AcASP2 <i>Onchocerca volvulus</i> : Ov-RAL-1 <i>Ascaris</i> spp: Asc s 1 (ABA-1) <i>A. simplex</i> : AsU22 <i>B. malayi</i> : Bm22U <i>Dirofilaria immitis</i> : Di22U <i>O. volvulus</i> : Ov22U <i>Schistosoma</i> spp: FABPc <i>Ascaris lumbricoides</i> : As l 13 <i>B. malayi</i> : Bm13 <i>O. volvulus</i> : Ov13 <i>S. mansoni</i> : SmGST <i>W. bancrofti</i> : WbGST <i>E. granulosus</i> : EA21 <i>Necator americanus</i> : Nec a cal 1 <i>O. volvulus</i> : OvRAL1
AF007 AF018	Venom Allergen-Like VALs SPX/RAL-2 proteins specific to nematodes SCP/TAPS (Sperm-Coating Protein/Tpx-1/Ag5/PR-1/ Sc7)	<i>Anisakis simplex</i> : Ani s 1 <i>E. granulosus</i> : antigen 5 <i>Trichinella spiralis</i> : TsSerP <i>S. mansoni</i> : SmSP1 <i>Anisakis simplex</i> : Ani s 4 <i>A. lumbricoides</i> : Al-CPI
AF208	Nematode polypeptides	
AF010	Glutathione S transferases	
AF038 AF055	Cyclophilins Calreticulins	
024	Serine protease inhibitors	
005	Cysteine protease inhibitors	

have been proposed as helminth vaccine targets. The Glutathione transferase As l 13 is one of at least six isoforms (Acevedo et al., 2013) and is structurally similar to mite and cockroaches GSTs (Mueller et al., 2015). Ani s 1 is a thermostable 24 kDa serine protease inhibitor, Ani s 4 is the *Anisakis* cystatin, a cysteine protease inhibitor; Ani s 7 is a valuable marker of infection because 100% of infected individuals have specific IgE against it. Na AS P2q shares structural homology with the PR1 family (proteins related to pathogenesis 1). Nacal-1 binds IgE from all subjects with antibodies to *Necator* extract. SmVAL4 is a member of the Venom-Allergen-Like proteins family. Sm22.6 belongs to the TAL family (Tegumental allergen-like).

3. Allergen sequences and accession numbers

Isoforms have been reported for Asc l 3, Asc s 1, and Ani s 4. They are included in Table 2.

4. Recombinant allergens

All the allergens included in this section have been cloned and obtained in several expression systems (Table 3).

5. Purified natural allergens

Several helminth allergens have been obtained in their natural forms to be used in experimental procedures. Natural and recombinant *A. suum* GST have been purified and their biological activities have been

Table 2
Allergen sequences (UniProt) and isoforms of helminth IgE binding components.

Allergen source	Allergen	Function	Isoforms	Immunological relevance	References
<i>A. lumbricoides</i>	Tropomyosin (Asc l 3)	Muscle contraction proteins	Asc l 3.0101 (G0L3K2) Asc l 3.0102 (A8DKY0)	Both isoforms induce cross reactive IgE to Blo t 10 and Der p10.	(Ahumada et al., 2015; Santos et al., 2008)
	Polyprotein ABA 1 (Asc s 1, Asc l 1)	Specific nematode polyprotein	Asc s 1.0101 (Q06811) Asc l 1 (Q9TY44)	Antibodies to ABA-1 is considered <i>Ascaris</i> resistance marker.	(McSharry et al., 1999)
	GST (Asc l 13)	Glutathione transferase	Asc l 13.0101 (P46436)	No evidence of IgE cross-reactivity with any allergen component in mites. 2D IgE immunoblotting revealed at least six GST isoforms in <i>A. lumbricoides</i> . This can be clinically relevant considering cross reactivity with mite GSTs.	(Acevedo et al., 2013)
	Ani s 1	Serine protease inhibitor	Ani s 1.0101 (Q7ZLK3)	Ani s 1 is the major and more potent allergen of this specie.	(Gamboa et al., 2012)
<i>A. simplex</i>	Ani s 4	Cysteine protease inhibitor	Ani s 4.0101 (Q14QT4)	Recombinant Ani s 4 activates basophils of subjects sensitized to <i>Anisakis</i> .	
	Ani s 7	NA	Ani s 4 (P83885) Ani s 7.0101 (A9XBJ8)	Ani s 7 induces IgE responses in all infected subjects; it has great diagnostic value as an infection and allergy marker.	(Anadon et al., 2009; Daschner et al., 2014)
	Ani s 9	NA	Ani s 9.0101 (B2XCP1)	Ani s 9 induces allergic inflammation in a respiratory murine model.	(Rodríguez-Pérez et al., 2008; Cho et al., 2014)
	SmVAL4	ES protein Larvae stage transition	NA	rSmVAL4 induces allergic asthma and passive skin anaphylaxis in mice.	(Farias et al., 2012)
<i>Schistosoma spp</i>	Sm22.6	Tegumental allergen like protein (TAL)	SmTAL1 (Q4TTW6)	Human IgE response to Sm22.6 is associated with partial immunity.	(Fitzsimmons et al., 2012; Wan et al., 2014)
	EF-hand domain				
	NaASP2	ES protein	Nec a ASP-2 (Q7ZIH1)	High levels of IgE against NaASP2 are associated with a lower risk of reinfection.	(Bethony et al., 2008; Diemert et al., 2012)
	Na cal 1	Larvae stage transition Calreticulin	Nec a cal 1 (O76961)	Na cal 1 induces basophil degranulation and histamine release.	(Pritchard et al., 1999; Pritchard et al., 2007)

Table 3
Biologic and structural characteristics of IgE binding helminth components.

Source	Allergen	Natural Recombinant	Post-translational modification	Available structure information	References
<i>A. lumbricoides</i>	Asc s 1 (ABA-1)	nABA-1 (parasite-derived material) rABA-1A (<i>E. coli</i> system)	NA	NMR 2XV9 data. ABA 1 is a small, monomeric globular protein, adopts a novel seven-helical fold comprising a long central helix that participates in two hollow four-helical bundles on either side. Discrete hydrophobic ligand-binding pockets are found in the N-terminal and C-terminal bundles, and the amino acid side chain is affected by ligand (fatty acid) binding.	(Meenan et al., 2011)
	GST Asc 113	nGSTA rGSTA (<i>E. coli</i> system)	Glycosylation suggested	The superposition of the predicted models for the tertiary structure of <i>Ascaris</i> GST1 with B10 t 8, Der p 8 and Bla g 5 identified several clusters of conserved and surface-exposed residues (patches) that could represent cross-reactive and species specific epitopes.	(Acevedo et al., 2013; Mueller et al., 2015)
<i>A. simplex</i>	Ani s 1	nAni s 1 rAni s 1 (<i>E. coli</i> system)	NA		(Kobayashi et al., 2008)
	Ani s 4	nAni s 4 rAni s 4 (<i>E. coli</i> system)	NA		(Rodríguez-Mahillo et al., 2007)
	Ani s 7	nAni s 7 rAni s 7 (<i>E. coli</i> system)	Glycosylated		
	Ani s 9	nAni s 9 rAni s 9 (<i>E. coli</i> system)	NA		(Rodríguez-Pérez et al., 2008)
<i>Schistosoma spp</i>	SmVAL4	rSmVAL4 (<i>Pichia pastoris</i> system)	N-glycosylated Asn118	X-ray diffraction data. SmVAL4 has the characteristic sandwich and central SCP/TAPS cavity, has only one of the signature CAP cavity tetrad amino-acid residues and the histidines that coordinate divalent cations such as Zn2 + in other SCP/TAPS proteins are missing. SmVAL4 has a cavity between -helices 1 and 4 that was observed to bind lipids in tabylsin-15, suggesting the ability to bind lipids.	(Kelleher et al., 2014)
	SmTAL1	nSmTAL1 rSmTAL1 (<i>E. coli</i> GST system)	NA	Intrinsic fluorescence and differential scanning fluorimetry data. SmTAL 1 consisted of an N-terminal, largely α -helical domain containing two EF-hand-like structures and are able to bind calcium ions reversibly.	(Thomas et al., 2015)
<i>N. americanus</i>	NaASP2	nNaASP2 rNaASP2 (<i>Pichia pastoris</i> system)	NA	X-ray diffraction data of the first three dimensional atomic view of an ASP. Na-ASP-2 is a representative single-domain ASP, comprised of an N-terminal loop, a PR-1 domain, and a short, cysteine-rich C terminus. The tertiary structure of the PR-1 domain is a three-layer $\alpha\beta\alpha$ sandwich, in which a three-stranded antiparallel sheet, lies between two layers of helices. The short C terminus is folded into a loop structure, followed by a helical turn, a short strand and terminating in a random coil.	(Asojo et al., 2005)

demonstrated (Hansen et al., 2016) (Table 3).

6. Post-translational modifications

Available data is in Table 3.

7. Available structure

The structures of Asc l 13 and ABA1 are available. There is a model for Asc l 3, Table 3.

8. Use in diagnosis

Two helminth allergens can influence the diagnosis of allergy: Asc l 3 (tropomyosin) and Asc l 13 (Glutathione transferase). Since there is evidence of potential clinical impact of Asc l 3 and other tropomyosins, in the Tropics sensitization to this allergen should not be considered as non-relevant cross reactive sensitization. On the contrary, given the possibility that some patients are co exposed to helminths and HDM, the IgE response to mite tropomyosins could be greater than when is induced only by HDM. In the tropics ABA-1 could be used for detecting genuine sensitization to *Ascaris*. However, none helminth allergen included is currently used in routine diagnosis of allergy.

9. Vaccine candidates, hypoallergenic derivatives

There is no vaccine candidate for preventing or treating allergy using helminth molecules; however, there are several for preventing helminth infections, some of them have elicited allergic symptoms when used. The recombinant NaASP2 produced in *Pichia pastoris* was tested as vaccine candidate; during Phase 1 trial the participants received intramuscular injections of the recombinant protein. No adverse effects were registered and IgG4 titers were detected after the second dose, however anti-NaASP2 IgE was detected at 14 days after the third injection (Bethony et al., 2008). The same authors analyzed the effect of NaAS-2 in 9 volunteers that had received anti helminthic treatment 35 days before vaccination. One hour after vaccination 3 participants had acute generalized urticaria and the trial was suspended. Importantly, levels of IgE to NaASP2 were 21 times higher in the subjects with urticaria compared to those observed in subjects without allergic reaction (26.5 vs 1.25 kU/L) (Diemert et al., 2012). Also, molecular modifications have been made on NaASP2. Zhan et al have succeeded in designing a chimeric human NaASP2-Fcγ fusion, which binds specifically to the FcγRIIb receptor and prevents the activation of FcγRI. The fusion protein was expressed in *P. pastoris* and tested in vitro for basophil histamine release and skin tests in dogs, finding an important reduction of allergic reactions. The maintenance of immunogenicity and efficacy as a vaccine in hamsters infected with L3 larvae of *N. americanus* was also demonstrated (Zhan et al., 2012).

10. Clinical trials

There are no clinical trials of allergy treatment or prevention using helminth molecules. As mentioned, clinical trials for testing anti helminth vaccines have been done. Also, there are trials using helminth infections as potential anti-inflammatories mainly in autoimmune diseases (Elliott and Weinstock, 2017), but the use of purified immunomodulatory molecules is still in preclinical phase using animal models.

11. Future directions

Defining the allergenic potential of helminth IgE binding antigens is a necessary starting point for several basic and clinical activities, among them:

- To evaluate their importance as adjuvants of allergy symptoms or diagnostic confounders. In the Tropics, where HDM and helminths are prevalent, the impact of cross reactivity and helminth non-specific immune stimulation remains to be fully defined. In addition, recent findings suggest that cross reactivity could go beyond HDM, also involving pollen components (Tyagi et al., 2015; Acevedo et al., 2016). More epidemiologic and experimental studies are needed to define the real impact of the IgE response to *A. lumbricoides* allergenic components on allergic symptoms. These studies would include the identification and mapping of pathogenic B and T cell epitopes of Asc l 3, Asc l 13 and ABA-1. Also, a better characterization of *A. simplex* allergens will help to understand the mechanisms and risk factors for anisakiasis.
- To select parasite vaccine as an alternative for controlling helminthiasis in endemic regions. For different reasons helminthiasis cannot be totally eradicated, which implies that other strategies, such as vaccination, are plausible alternatives. Vaccine candidates should be tested for their allergenic activity in order to prevent allergic adverse reactions. The knowledge of the general spectra of biological and immunological properties of helminth IgE binding components will help to avoid unnecessary trials using allergenic molecules.
- To choose parasite immunomodulatory molecules to be tested for treating allergy and autoimmunity. The field of helminth derived immunomodulation is increasing and a great number of promissory molecules have been obtained (Smallwood et al., 2017; Coronado et al., 2017; Coronado et al., 2015), but the study of their allergenic activity remains to be done for most of them, which could limit their clinical use in humans.
- To better understand the molecular basis of natural induced IgG isotypes together with IgE responses. During the last years several groups have rediscovered the role of IgG, particularly IgG4, as a natural and physiologic response to common allergens exposure (Hofmaier et al., 2015; Holt et al., 2016). Since this type of antibody response is also observed during several chronic helminth infections, it could be interesting to identify shared aspects between those common and helminth allergens that bind IgE but not induce allergy inflammation.
- To explore the genetic and evolutionary basis of allergenicity and allergenic activity, two complex traits close related to the immune responses against common allergens. Future comparative genomic and immunogenetics research will help to evaluate whether the allergic response is a legacy of the immune responses to helminths or is a trait that has evolved independently of the selective pressure of infectious diseases.

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