

Helminths and allergy: the example of tropomyosin

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Parasitic worms contain potent allergens, but epidemiological and experimental studies suggest that infections with certain helminths are negatively associated with the prevalence of allergic diseases. This seeming contradiction can be addressed by using filarial tropomyosin as an example. This protein shares structural features and crossreacting B-cell epitopes with other highly allergenic invertebrate tropomyosins. Nevertheless, it usually does not provoke allergic disease in infected individuals. In addition, it is one of the most prominent candidates for an anti-nematode vaccine. Recent data suggest mechanisms that might prevent hosts from developing allergic reactions against allergens of their parasites, such as filarial tropomyosin.

Allergic reactions and helminth infections

Epidemiological studies and experiments in animal models suggest that chronic infections with certain helminths can alleviate allergic diseases [1,2]. At first glance, this seems to be a paradox in view of the fact that parasitic worms contain potent allergens and confront the host immune system with such components. For example, it is known that the handling of the body fluids of *Ascaris* by researchers can lead to fulminant allergic reactions [3]. How can helminth infections at the same time have an anti-allergic effect? This question has been investigated using as an example tropomyosin, a highly conserved muscle protein that is consistently described as a ‘pan-allergen’ – that is, a widely occurring potent allergen. A closer look at the properties of nematode tropomyosin provides some answers but also raises new questions.

Infections with nematodes and other helminths are regularly associated with eosinophilia and high levels of IgE, signs that are usually found in patients with type I hypersensitivity reactions (usually termed ‘allergy’ or ‘atopy’) against certain antigens. Sensitization with such allergens results in T helper cell type 2 (Th2) responses, which support the expression of interleukin (IL)-4 and IL-5, leading to the production of IgE by plasma cells and the differentiation of eosinophils. Binding of the Fc portion of IgE antibodies to the Fcε receptor (FcεR) on the surface of basophils and mast cells results in the sensitization of these cells. After sensitization, allergic effector mechanisms can be triggered by antigen contact, when surface-bound IgEs are crosslinked by the allergen (Figure 1). This engagement of the FcεR leads to degranulation of basophils or mast cells. Release of histamine and other mediators,

and subsequent attraction and activation of effector cells (mostly eosinophils), leads to Th2 inflammatory responses that can kill parasites but can also result in damage to the host tissue. Much of this damage is ascribed to the aggressive constituents of eosinophil granules that are discharged in the effector phase of allergic inflammation [4].

It is conceivable that Th2 responses in worm-infected individuals are induced by parasite components that are either secreted by living worms or released when dead parasites disintegrate. Nematode infections continuously confront the host with a large number of different antigens, among which are allergens. However, these allergens do not usually induce allergic responses. This is particularly evident in the case of filarial infections, where the adult worms living within the tissues of the host reach life spans of more than a decade and constantly release large amounts of microfilariae. After several months, the larval stages die and degrade. The skin of patients heavily infected with *Onchocerca volvulus* harbors on average 12 million microfilariae, in addition to 20–50 adult worms [5]. This number highlights the substantial amounts of foreign material that a patient’s immune system must deal with.

In the context of helminth infections, eosinophils or macrophages can efficiently attack certain IgE-opsonized helminth stages – for example, larval stages of schistosomes or nematodes by antibody-dependent cellular cytotoxicity (Figure 1). However, many adult worms exploit their hosts successfully in spite of eosinophilia and high IgE levels, suggesting that the cells and antibodies are prevented from being active against these parasites [6]. The fact that worm-infected individuals suffer less from allergies than do uninfected ones has led to the assumption that well-adapted parasites – although strongly inducing Th2 responses – prevent the triggering of allergic effector mechanisms, and thus avoid host defenses [7,8]. Reactions against environmental allergens would be blocked simultaneously as a side effect, resulting in a lower prevalence of allergic disease.

Blockage of allergic effector mechanisms by nematode infections

Several anti-allergic mechanisms have been described, from various nematode infections, which might cooperate in a given host. First, nematode infections induce high levels of ‘blocking antibodies’ of the IgG4 subclass. These antibodies compete with specific IgE for epitopes, thus reducing the probability that sensitized mast cells and basophils can bind to a given antigen with their surface-located IgE [9].

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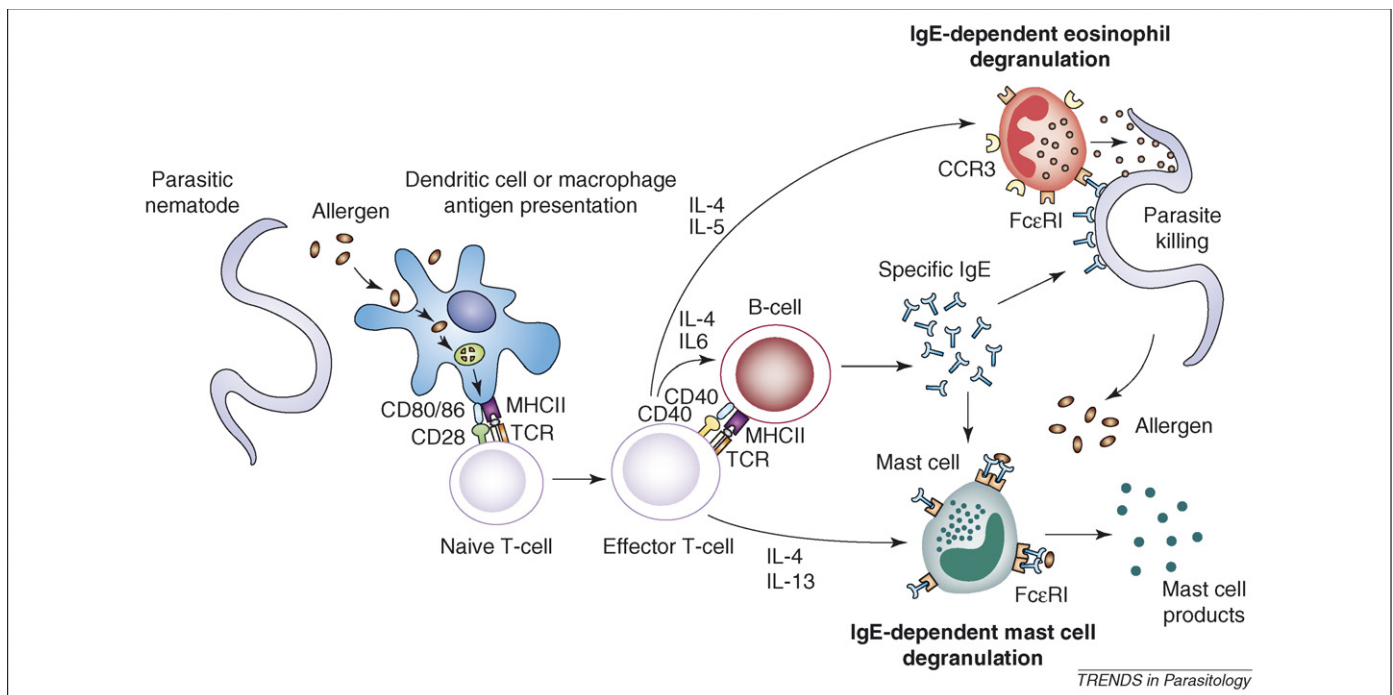


Figure 1. Induction of IgE-mediated anti-helminth effector reactions. Parasitic nematodes release allergens that are presented to naïve T cells by dendritic cells, macrophages or B cells. Activated T cells (effector T cells) release cytokines that activate other cells of the immune system and among others induce production of allergen-specific IgE by B cells. Allergens released by the nematodes can crosslink specific IgE on the mast cell surface and initiate their degranulation. Specific IgE bound to the surface of the parasites can be recognized by eosinophils and initiate their degranulation, eventually leading to parasite killing and tissue damage. Abbreviations: CCR3, CC chemokine receptor 3; TCR, T cell receptor; FcεRI, receptor I for Fc portion of IgE; CD80/86, CD28, CD40 and CD40L are co-stimulatory molecules.

Furthermore, nematode infections have been shown to upregulate nonspecific IgE, which could lead to a dilution of specific IgE molecules and reduce the degranulation of mast cells or basophils. However, this concept has been challenged recently [10]. Second, it has been demonstrated that chronic nematode infections, such as with the murine intestinal nematode *Heligmosomoides polygyrus*, induce regulatory T cells that suppress allergic responses against the model allergen ovalbumin [11]. Third, nematode infections induce the production of anti-inflammatory cytokines (IL-10 and transforming growth factor-β) from various cell sources, which interfere with allergic effector functions [12,13]. Finally, nematodes release products that inhibit the chemoattraction and activation of effector cells – for example, by degrading eotaxin, a chemoattractant and stimulator of eosinophils [7,14]. All of these mechanisms result in a staggering coexistence of high levels of IgE and circulating eosinophils, with an absence of Th2 inflammatory responses [15].

Tropomyosin and other nematode allergens

The molecular basis for the allergenicity of a protein remains largely unknown, and the allergenic potential of a given protein cannot be reliably predicted. The mechanisms leading to the development of Th2 responses against an antigen seem to be default pathways that function in the absence of strong Th1-inducing stimuli [16], such as Toll-like receptor ligands [17,18]. Consequently, many different types of antigen can be allergenic. One of the allergens present in all helminths is tropomyosin, a muscle protein that is highly conserved throughout the animal kingdom. Invertebrate tropomyosins account for widespread allergic reactions against seafood, house dust mites

and cockroaches [19]. Tropomyosin also induces reactive IgE in nematode infections [20–23]. Due to this crossreactivity, invertebrate tropomyosin is considered to be a pan-allergen. Interestingly, vertebrate tropomyosins are not allergenic [24].

Some helminth allergens, such as nematode polyprotein allergens (NPAs) [25], are well described. These are high molecular weight proteins consisting of repetitive subunits, which include *Ascaris* body fluid antigen 1, an NPA from *Ascaris suum* or *Toxocara canis* body fluid antigen, and orthologues from other intestinal nematodes and from filariae (gp15/400 from *Brugia malayi* or LL20 from *Loa loa*). NPAs are lipid-binding proteins with a suspected immunomodulatory function. Furthermore, allergens of parasitic nematodes include a 31 kDa protein from *Trichostrongylus colubriformis* [26], a high molecular weight allergen from *Teladorsagia circumcincta* [27] and a 31 kDa allergen from *Strongyloides stercoralis* [28]. Interestingly, nematode allergens also include paramyosin from *Anisakis simplex* [29], a protein that shares many features with tropomyosin.

Structure and function of tropomyosin

Tropomyosin is a microfilament-associated protein present in all eukaryotic cells. It is found in multiple isoforms that are characteristic for specific types of tissue [30]. The most common isoform of tropomyosin has a molecular weight of ~32.5 kDa and is abundant in muscle, where it forms dimers of ~20 Å in diameter and 400 Å in length in the form of a coiled-coil rod with two polypeptides swirling one around the other [31]. In thin filaments, these dimers are associated in a head-to-tail manner and form overlapping chains that run along actin filaments (Figure 2). They

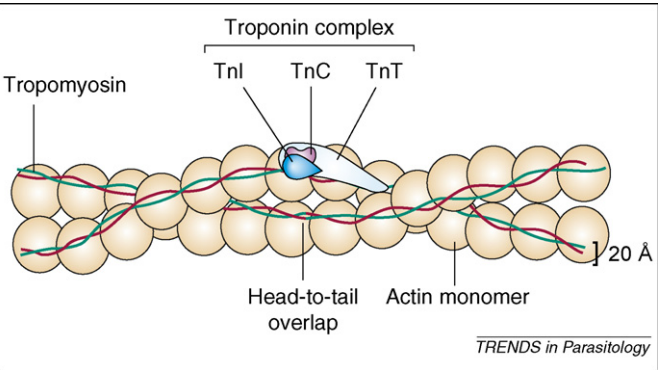


Figure 2. Structure of tropomyosin in the context of a thin muscle filament. Tropomyosin thin filaments consist of two helical proteins swirled around each other. The tropomyosin strand is arranged along an actin filament. In the resting stage, tropomyosin covers myosin-binding sites of actin. Ca^{2+} and the proximity of myosin heads lead to activation of troponins TnI, TnC and TnT, which start a cyclic reaction resulting in a shift of tropomyosin by 35° . This reversible shift exposes the hitherto blocked myosin-binding sites and enables myosin heads to bind to actin, a prerequisite for locomotion. Modified, with permission, from Ref. [63].

have a crucial role in the process of thin filament activation by blocking or freeing actin-binding sites in the presence of calcium ions and myosin [32]. In this respect, tropomyosin is essential in the process of muscle work, proper action of the movement apparatus and the basic functionality of filaments within the cytoskeleton. Activation of tropomyosin is calcium dependent, and is controlled by a complex of three troponins [33]. The coiled-coil conformation of tropomyosin is a result of the architecture of the protein, which is built from heptapeptides. This motif is made up of a sequence of amino acids (aas) with a similar chemical nature, resulting in imperfect repeats of short aa sequences [34]. Both the N- and C-termini of tropomyosin have conserved aa residues, which are vital for the creation of ‘head-to-tail overlaps’ [35].

The complex interaction with other proteins involved in the process of movement or transport enables only a low level of freedom in the aa composition of tropomyosin. Therefore, it is not surprising that the degree of homology is high among tropomyosins, even of phylogenetically distant species [30]. The homology of aa sequences of tropomyosins originating from invertebrate and vertebrate organisms reaches over 50%. The sequence identity between tropomyosin isoforms of closely related invertebrate species can reach 95%, as, for example, between the orthologues of the human pathogenic filarial nematode *O. volvulus* and the rodent filarial *Acanthocheilonema viteae* [21]. Detailed studies of a muscle isoform of vertebrate tropomyosin identified highly conserved actin-binding aa motifs in certain positions within the heptapeptide repeats throughout the animal kingdom [36].

Tropomyosin isoforms in nematodes

The free-living nematode *Caenorhabditis elegans* has one tropomyosin gene (*tmy-1* or *lev-11*), which is located on chromosome I, spans 14.5 kb and includes 14 exons. Alternative splicing of its transcript leads to the creation of four different tissue-specific isoforms [37,38]. RNA interference (RNAi)-mediated gene silencing revealed that tropomyosin is essential for body morphology, coordinated

movement and embryogenesis [38]. The high degree of conservation of tropomyosins suggests that these proteins in parasitic nematodes have similar functions and architecture. Thus, in recent years tropomyosin has also become a target gene for RNAi experiments in parasitic worms such as *T. colubriformis*, *Haemonchus contortus* and *Ostertagia ostertagi* [39].

Interestingly, tropomyosin from parasitic worms was not only found to be abundant in the muscle layer, but was also described to occur in the cuticle of *O. volvulus* [22] and *Trichinella spiralis* [40]. Whether this cuticle type of tropomyosin represents a particular isoforms, and what specific functions it might have, is not known.

Allergenicity and crossreactivity of invertebrate tropomyosins

Interestingly, vertebrate tropomyosins were reported to be non-allergenic in humans [24], probably owing to the high homology among vertebrate tropomyosins, preventing recognition as foreign entities. By contrast, particular regions of invertebrate tropomyosins that differ widely in structure from the corresponding stretches of vertebrate tropomyosin induce IgE antibodies and are thus potent allergens for humans [41]. Tropomyosin from parasitic nematodes is no exception in this regard and induces IgE in infections of humans and experimental animals [20–22]. Because of the similarity of invertebrate tropomyosins, the tropomyosin-specific IgE antibodies cross-react widely, meaning that they recognize tropomyosins from other species. Thus, individuals with a shrimp allergy might also be allergic to other crustaceans, and their antibodies might even crossreact with tropomyosins from more distantly related invertebrates owing to recognition of shared IgE epitopes (Table 1). Thus, IgE against tropomyosin from shrimp crossreacts with tropomyosin from house dust mites and insects such as cockroaches, grasshoppers and fruit flies [42]. Tropomyosin is therefore considered to be a cause for widespread crossreactivity, particularly among seafood allergens and aeroallergens of invertebrate origin [19].

Table 1. Crossreactivity of tropomyosins from different invertebrate species

IgE-inducing tropomyosin	Crossreactive tropomyosin	Refs
<i>A. simplex</i> (nematode)	House dust mite	[60]
<i>O. volvulus</i> (nematode)	Shrimp	[22]
<i>P. aztecus</i> (shrimp)	Lobster, cockroach, grasshopper, fruit fly, house dust mites, silverfish	Reviewed in [42], [61]
<i>Homarus americanus</i> (lobster)	Other lobster, shrimp	Reviewed in [42]
<i>Charybdis feriatius</i> (crab)	Lobster, shrimp	Reviewed in [42]
<i>Periplaneta americana</i> (cockroach)	Shrimp	Reviewed in [42]
<i>Blattella germanica</i> (cockroach)	Other cockroach, mites	[62]
Mollusk	Shrimp, snail	Reviewed in [42]

This crossreactivity extends to nematode tropomyosin [22,43]. Thus, human onchocerciasis sera contain anti-tropomyosin antibodies, which can be completely absorbed with shrimp tropomyosin [22]. Similarly, tropomyosins from shrimp, cockroach or house dust mite share epitopes with tropomyosin from *A. simplex*, a parasitic nematode sporadically transmitted by infected fish and known as 'sushi worm' [20]. This crossreactivity can lead to false-positive serological tests; for example, a person with shrimp allergy can be misdiagnosed to be infected with *A. simplex* [23].

IgE epitopes of tropomyosin

One trait common to many allergens is the repetition of structural elements within the molecule, which results in repeated identical or similar epitopes. Such repeated epitopes (Figure 3) can simultaneously crosslink several IgE molecules, leading to efficient aggregation of FcεR on the surface of sensitized basophils and mast cells, and thus to efficient degranulation [44].

So far, several IgE regions have been characterized on shrimp tropomyosin, a well-studied invertebrate model allergen, and on tropomyosins from several other invertebrates [45]. The majority of IgE epitopes are clustered in five regions of the protein that are responsible for the binding of the two tropomyosin helices to each other. The sequences are relatively conserved in invertebrates – for example, among shrimp, cockroach and house dust mite tropomyosins [46,47] (Figure 3). However, they show lower sequence homology with the corresponding domains

of vertebrate tropomyosins and thus constitute B cell epitopes. The homology between invertebrate tropomyosins extends also to parasitic nematodes such as *A. simplex*, *A. viteae*, *O. volvulus*, *T. colubriformis*, *A. suum* and *T. spiralis* [21,22,40,48,49].

Ayuso *et al.* [45] showed that five major IgE-binding regions of shrimp tropomyosin contain an aa sequence characterized by a concentration of negatively charged aas, forming the LEXXL motif, where L is leucine and X is usually an acidic aa such as glutamic acid (E) or aspartic acid (D). These domains are probably hidden within the coiled coil because they mediate binding between the two coils [50]. Recognition of these epitopes by IgE would require the exposure of the motifs, either by high flexibility of the tropomyosin dimer, by denaturation, or by degradation. It is likely that additional IgE epitopes of tropomyosin exist because the usually performed epitope mapping with synthetic peptides detects only the minor fraction of sequential epitopes, whereas most B cell epitopes are conformational and their structure cannot easily be mirrored by synthetic peptides. Tropomyosin also has repeated actin-binding domains. However, being highly conserved, even between invertebrates and vertebrates, these domains are not immunogenic.

T cell responses against tropomyosin counteract antibody responses

Invertebrate tropomyosin not only induces B cell responses, but is also capable of inducing strong T cell

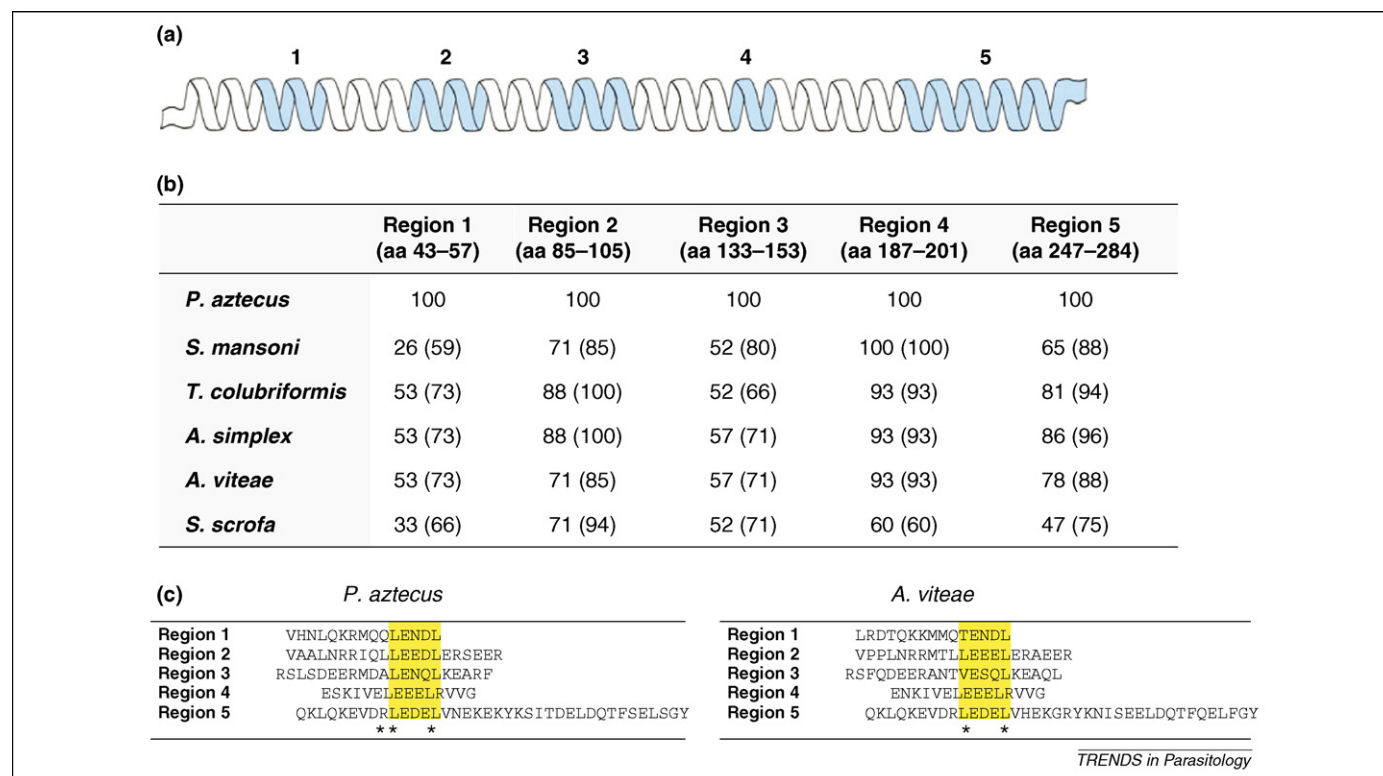


Figure 3. The molecular basis of crossreactivity between invertebrate tropomyosins. Immunodominant IgE regions of shrimp (*Penaeus aztecus*) tropomyosin. (a) Regions from 1 to 5, as described by Ayuso *et al.* [45], are marked in blue. (b) Percent of identity (first number) and similarity (based on the presence at the same position on two different tropomyosin molecules of aas that share similar chemical properties – e.g. basic, acidic, polar or non-polar) of aa (second number) between IgE-inducing regions in tropomyosin sequences of several species as compared with *P. aztecus*. (c) Alignment of IgE-inducing regions of *P. aztecus* tropomyosin compared with the alignment of corresponding regions from *A. viteae*. GenBank accession numbers of sequences: *P. aztecus* DQ151457, *Schistosoma mansoni* A60607, *A. viteae* AF000607, *T. colubriformis* J04669, *A. simplex* Y19221, *Sus scrofa* X66274. Asterisk, identical aa.

responses. T cells of mice that had been immunized with shrimp tropomyosin proliferated strongly after restimulation with a synthetic peptide, spanning residues 261–281 at the C-terminal end of the protein. The peptide-induced proliferation of T cells was as strong as with the complete protein, revealing strong T cell epitope(s) in the C-terminus [51]. Interestingly, administration of this peptide along with the Th1-inducing adjuvant Freund's complete adjuvant led to significant downregulation of strong tropomyosin-specific IgG and IgE responses in mice that had previously been immunized with native tropomyosin emulsified in alum [51]. Such a modulation of the antibody response against allergens, potentially brought about by alteration in cytokine profiles, can abrogate allergic reactions, a principle applied for specific immunotherapy of allergy patients [52]. This underlines that the allergenic properties of a protein depend greatly on the cytokine environment.

Host-protective properties of nematode tropomyosin

Nematode tropomyosin is one of the few antigens described to induce significant protective immunity against parasite challenge in vaccination studies (Table 2). Does this mean that allergens could be particularly suitable vaccine candidates? Immunization with native tropomyosin protected against *T. colubriformis* L3 challenge in a guinea-pig model, reducing worm burdens by 43%–51% [53]. Immunization of BALB/c mice with a C-terminal fragment of *O. volvulus* tropomyosin (MOv14) comprising 136 aas and expressed as a maltose-binding protein (MBP) fusion evoked 48%–62% reduction in the number of *Onchocerca lienalis* microfilariae. In this model, the challenge infection consisted in microfilariae of a cattle parasite intravenously inoculated into mice [54]. Interestingly, tropomyosin-associated protection against such microfilariae could be transferred with serum to naïve recipient BALB/c mice, so the protection is clearly based on antibodies [55]. Immunization with MOv14 also protected jirds against a challenge with L3 of the rodent filaria *A. viteae*, reducing adult worm burdens by 46% and the level of circulating microfilariae by up to 89% [54]. Vaccination of mice with full-length cDNA of *O. volvulus* tropomyosin through the

intramuscular and epidermal (Gene Gun) route resulted in 20% protection against challenge with *O. volvulus* [56]. The earlier-mentioned vaccination studies relied on atypical parasite–host associations or heterologous tropomyosins for vaccination. However, testing of the homologous protein in a natural parasite–host association corroborated the protective capacity of this protein. Immunization of jirds with native *A. viteae* tropomyosin induced up to 64% protection against challenge infection with L3, as shown by a reduction in worm burdens, with a corresponding 93% reduction in circulating microfilariae [57].

Comparative studies in the jird model showed that the protective effect of tropomyosin against challenge with *A. viteae* L3 clearly depends on the conditions of immunization [21]. Immunization with recombinant *A. viteae* tropomyosin together with the STP adjuvant (an adjuvant based on a mixture of squalane, Tween and pluronic) or vaccination with its cDNA – both inducing predominantly Th1 responses – resulted in protection of jirds in terms of reduction of worm burdens. By contrast, immunization with recombinant or native tropomyosin together with alum (a scheme inducing Th2 responses that correspond to the natural context of this filarial infection) did not protect against challenge infection [21]. These data suggest that the nematode parasites were vulnerable to a type of host defense reaction that does not usually develop during natural infections. Conspicuously, tropomyosin-induced protection against L3 was negatively associated with antibody responses in these experiments. This shows that the effector mechanisms in this constellation were not antibody dependent, suggesting a role for Th1 inflammatory responses as the mechanism of protection. Thus, tropomyosin was protective against L3 challenge only when the conditions of immunization counteracted its allergenic properties. It is conceivable that this type of vaccination relies on the fact that the worms are not able to evade Th1 inflammatory responses, whereas they successfully avoid defense mechanisms of the Th2 type to which they are usually exposed during infection.

The seeming contradiction between tropomyosin-induced antibody-mediated protective immunity against

Table 2. Vaccine studies with tropomyosin of various helminths^a

Parasite	Host	Vaccine formulation	Reduction in worm burden (%)	Comments	Refs
<i>T. colubriformis</i>	Guinea pig	41 kDa fraction of L3 extract	43–51 (adults)		[53]
<i>O. volvulus</i>	(i) BALB/c mouse (ii) Jird	C-terminal fragment + MBP (Ov14)	48–62 (Mf) 46 (adults) 89 (Mf)	(i) Ov14 used against <i>O. lienalis</i> challenge (ii) Ov14 used against <i>A. viteae</i> challenge	[54]
<i>O. lienalis</i>	BALB/c mouse	Aqueous extract of Mf immune serum transfer	51 (Mf) 54 (Mf)		[55]
<i>O. volvulus</i>	BALB/c mouse	Full-length cDNA	20 (L3)	i.m. and epidermal (Gene Gun)	[56]
<i>A. viteae</i>	Jird	Native tropomyosin	40–64 (adults) 93 (Mf)		[57]
<i>A. viteae</i>	Jird	a) Full-length tropomyosin b) Full-length tropomyosin c) Native tropomyosin d) Full-length tropomyosin cDNA	0 (adults) 35 (adults) 29 (adults) 28 (adults) 43 (adults)	s.c. with alum s.c. with STP s.c. with STP i.m. with PBS i.m. with AdjuPhos	[21]
<i>S. japonicum</i>	BALB/c mouse	Native protein	21 (adults) 30 (eggs)		[58]

^aAbbreviations: AdjuPhos, aluminum phosphate gel adjuvant; alum, aluminum hydroxide fluid gel; i.m., intramuscular; Mf, microfilariae; s.c., subcutaneous.

Review

microfilariae [54,55] and antibody-independent protective responses against L3 [21] could be due to differences between the larval stages regarding their susceptibility to these immune effector mechanisms. Interestingly, the protective effect of tropomyosin is not restricted to nematode infections, in that immunization with native tropomyosin from *Schistosoma japonicum* was reported to induce protection against cercarial challenge (21% reduction in worm numbers, 30% reduction in egg recovery) [58], suggesting that the protective potential of this protein deserves attention.

Concluding remarks

The earlier-mentioned features of tropomyosin exemplify that endoparasitic nematodes and other helminths contain allergens as integral components, and continuously confront their hosts with these antigens. However, helminth infections can result in a decrease in allergic disease, even in the presence of hallmarks of allergic reactions, such as eosinophilia and high levels of IgE. This constellation suggests that the basic elements necessary for an allergic reaction are present, whereas key effector mechanisms are blocked by the worms. It is likely that this immune evasion relies on at least two mechanisms. First, in an indirect manner, helminth infections modulate the overall immune reactivity, leading to the formation of 'blocking antibodies', high levels of unspecific IgE and regulatory T cells [9–13]. Parasite components causing altered immune regulation are emerging [7], although the exact mechanisms have not yet been identified. Second, secreted helminth products seem to interfere specifically with certain steps in the activation of effector cells involved in allergic reactions. An example for this strategy is the recently described inhibition of IgE-mediated mast cell responses through an atypical engagement of Toll-like receptor 4 by ES62, a secreted glycoprotein of a filarial nematode [59]. It is suggestive that the earlier-mentioned anti-allergic effects of parasites are systemic and therefore also extend to responses against unrelated allergens, such as environmental allergens, in the sense of a bystander effect. In addition, it would be useful to study whether helminth allergens such as tropomyosin have specific traits that lead to less efficient allergic effector mechanisms. It would also be interesting to learn in more detail about the molecules that parasites have evolved to manipulate the effector mechanisms of their hosts against the allergens they produce. With this information, new approaches towards the understanding and management of allergic diseases could be developed.

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