



Characterization of the allergen filarial tropomyosin with an invertebrate specific monoclonal antibody

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ABSTRACT

Tropomyosins of invertebrates are pan-allergens responsible for wide spread allergic reactions against seafood and arthropods. As invertebrate tropomyosins are highly conserved, helminth tropomyosins are likely to show properties similar to these medically important allergens. Studies with a monoclonal antibody, NR1, raised against tropomyosin of the rodent filarial nematode *Acanthocheilonema viteae* revealed a B cell epitope common to helminths and marine mollusks, which does not occur in vertebrate tropomyosin. This antibody detected tropomyosin of *A. viteae*, other filariids, nematodes, trematodes and a cestode, and recognized as well tropomyosin of oyster, squid and octopus, but not of arthropods and vertebrates. Immunohistological analyses of *A. viteae*, *Onchocerca volvulus* and other nematodes using NR1 showed that tropomyosin is located in the fibrillar part of the body wall muscles and the uterus, and is also conspicuous in muscles of the pharynx, the vagina and other organs of the nematodes. The abundance of a pan-allergen like tropomyosin in parasitic worms and the counterintuitive, but well documented protection against allergic reactivity by some chronic helminth infections is discussed.

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1. Introduction

Parasitic worms continuously confront their hosts with immunogenic components, among others with proteins that are either secreted or are released when dead parasites are decomposed (Hewitson et al., 2008; Nagaraj et al., 2008; Smith et al., 2009). One of these molecules is tropomyosin, a conserved actin-associated muscle protein (Sereda et al., 2008). Invertebrate tropomyosin is one of the most important food allergens and has been described as a pan-allergen, meaning that tropomyosin from a given invertebrate species can induce potent cross-reactive IgE antibodies that recognize tropomyosins of other species as well. Invertebrate tropomyosin accounts for widespread allergic reactions for example against seafood, house dust mites and cockroaches (Reese et al., 1999; Lehrer et al., 2002). The allergenicity of invertebrate tropomyosin is due to the fact that, despite an

overall homology with the vertebrate protein, the molecule bears invertebrate specific epitopes that frequently induce IgE antibodies, as exemplified for shrimp tropomyosin (Ayuso et al., 2002). In contrast, IgE responses against tropomyosins of several vertebrate species are very rare (Ayuso et al., 1999), suggesting that vertebrate tropomyosins are usually not recognized as foreign components due to the high sequence homology between vertebrate tropomyosins.

Helminths do not only contain tropomyosin, but also a variety of other allergens, among others highly allergenic polypeptides (Kennedy, 2000), protease inhibitors (Shaw et al., 2003) and fatty acid binding proteins (Orton et al., 2007). Interestingly, due to such allergens individuals chronically infected with helminths show high levels of serum IgE and eosinophilia, but the prevalence of allergic skin reactivity is lower than in non-infected persons (Smits and Yazdanbakhsh, 2007). Several reports suggest that the absence of allergic effector mechanisms is an effect of immunomodulating proteins released by the parasite (Melendez et al., 2007; Schnoeller et al., 2008; Park et al., 2009). However, it cannot be excluded that in addition particular qualities of helminth allergens contribute to reduce allergic disease. As a step towards a better understanding we studied filarial tropomyosin, using among others a new monoclonal antibody specific for invertebrate tropomyosin.

Abbreviations: L3, third stage infective larvae; mf, microfilaria; mAb, monoclonal antibody.

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2. Materials and methods

2.1. Preparation of extracts and antigens

Muscle tissue was prepared from various vertebrates and invertebrates and minced in PBS pH 7.4 (containing a cocktail of protease inhibitors) on ice with a fine pair of scissors, homogenized in a glass homogenizer (Braun, Melsungen, Germany), and sonicated 4 times 30 s with 30 W using a microtip. Samples were centrifuged at $10,000 \times g$ for 15 min and the protein concentration in the supernatant was determined with the Bradford test. Aliquots of extracts were stored at -20°C until use.

Worm-derived tropomyosin was obtained by electroelution of a 43 kD band from SDS-PAGE-separated total extracts of *Acanthocheilonema viteae* female worms that was recognized by sera of mice immunized with recombinant tropomyosin of *Acanthocheilonema viteae* expressed in *E. coli* (rAvTropo, Hartmann et al., 2006).

2.2. Production of monoclonal antibodies (mAbs)

mAbs against *A. viteae* tropomyosin were produced according to standard procedures. Briefly, BALB/c mice were immunized 3 times with 25 μg worm-derived tropomyosin. The antigen was administered with 1:1 (v/v) alum (2% aluminium hydroxide; Reheis, Dublin, Ireland) as an adjuvant. Spleen cells of immunized animals were fused with mouse X63-Ag8 myeloma cells. Hybridomas were selected with HAT, supplemented with 25 U/ml IL-6 and resulting cells were screened for antibodies against worm-derived tropomyosin and rAvTropo. Positive hybridomas were subcloned at least 3 times. Clones NR1 (IgG1) and N11 (IgM) were used for further studies. A commercially available mAb against chicken gizzard tropomyosin (TM311, IgG1, Sigma) and mAbs against *A. viteae* chitinase (24-4, IgG1, Adam et al., 1996) and against a surface-associated epitope of *A. viteae* (1E5, IgM) served as controls.

2.3. Western blot, ELISA and epitope mapping

Methods were performed according to standard procedures. For Western blots, worm extracts in sample buffer were separated by SDS-PAGE in 12% gels under reducing conditions and proteins were blotted onto nitrocellulose membranes. These were incubated with undiluted hybridoma culture supernatant or with a 1:400 dilution of mAb TM311 after blocking and washing. Immunostaining was with anti-mouse IgG or IgM conjugated to alkaline phosphatase (Rockland, Gilbertsville, PA, USA).

ELISA was performed using undiluted hybridoma culture supernatant as a source of primary antibody and peroxidase-labeled goat anti-mouse IgG or IgM (Jackson ImmunoResearch, West Grove, PA, USA) in a dilution of 1:3000 as secondary antibody for immunostaining. To determine the specificity of mAbs, we first calibrated protein extracts of various invertebrates and vertebrates for a similar amount of protein present by visual inspection in the tropomyosin band in SDS-PAGE gels and immunoblots. Amounts of protein extracts that would result in a quantity of tropomyosin comparable between different samples were then coated onto ELISA plates. Deglycosylation of proteins was performed by exposing 96-well plates coated with 200 ng/well of worm-derived tropomyosin or total worm extract to 30 mM of Na-metaperiodate in Na-acetate buffer (pH 4.5) for 1 h at room temperature in the dark. After washing with 50 mM Na-acetate, wells were incubated for 30 min with 50 mM of Na-borohydride in PBS. After triple washing the plates were used for ELISA as described above.

SPOT technology (Kramer et al., 1999; Volkmer, 2009) was applied to map the target epitope of NR1. Briefly, rAvTropo was

synthesized as an array of overlapping 15-mer peptides with a shift of three amino acids on a cellulose membrane (Whatman 50, Maidstone, UK) according to the standard SPOT synthesis protocol (Wenschuh et al., 2000). The peptide arrays were blocked for 3 h with TBS blocking buffer (10% blocking-reagent, CRB, Norwich, Great Britain, and 5% sucrose in TBS buffer pH 8) and subsequently incubated with hybridoma culture supernatant diluted 1:1000 in TBS blocking buffer overnight at 4°C . Binding was detected and visualized with a secondary peroxidase-labeled anti-mouse IgG antibody (A-5906, Sigma–Aldrich). An incubation period of 2 h at room temperature and a dilution of 1:1000 were used. Binding was visualized using a chemoluminescence substrate (Pierce, Bonn, Germany) and the Lumi-Imager™ (Roche Diagnostics, Mannheim, Germany). As a control antibodies TM311 and 24-4 were used.

2.4. Immunohistology

Adult *A. viteae* and microfilariae (mf) were collected from experimentally infected *Meriones unguiculatus* and infective larvae (L3) from the tick *Ornithodoros moubata*. Fifty-eight onchocercomas from 47 patients in Liberia, Uganda, Ghana, and Mexico were included from the nodule material of previous studies (Büttner et al., 1988; Fischer et al., 1993; Hoerauf et al., 2003). Skin biopsies from mf carriers had been taken during nodulectomies in Uganda. The nodules had been surgically removed from the patients using local anaesthesia and aseptic conditions. Nodulectomies for research purposes had been approved by the Ethics Commission of the Medical Board in Hamburg and the authorities in the endemic countries. The procedures used were in accordance with the Declaration of Helsinki (1975 and its revisions in 1983, 2000 and 2002). Paraffin blocks with other worms were available from the helminth collection of the Bernhard Nocht Institute (Table 2).

Onchocercomas and worms selected for this study had been fixed in 70% ethanol since the mAb NR1 did not react with the worm muscles after formaldehyde fixation. The samples were embedded in paraffin using standard methods. The alkaline phosphatase anti-alkaline phosphatase (APAAP) technique was applied as recommended by the manufacturer (DakoCytomation, Hamburg, Germany). As primary antibody for the labelling of tropomyosin a 1:200 to 1:2000 dilution of affinity purified mouse mAb NR1 or undiluted hybridoma culture supernatant, respectively containing mAb N11 were used. An anti-mouse rabbit antibody (DakoCytomation) was applied as secondary antibody, Fast Red TR salt (Sigma, Deisenhofen, Germany) as chromogen and haematoxylin (Merck, Darmstadt, Germany) served as the counter stain. Negative controls included the incubation with serum albumin (0.1%, w/v) or with irrelevant antibodies instead of the anti-tropomyosin antibodies.

3. Results

3.1. Reactivity of anti-tropomyosin mAbs

To study properties of filarial tropomyosin, we produced mAbs against worm-derived tropomyosin of adult female *A. viteae*. One of the resulting mAbs, NR1 (IgG1), recognized a 43 kD band in immunoblots of male and female worms, microfilariae and L3 (Fig. 1A). NR1 in addition recognized rAvTropo, indicating that the target epitope was of protein nature. Another monoclonal antibody of this series, N11 (IgM), recognized the 43 kD band in worm extracts, but not rAvTropo (Fig. 1B), while isotype specific control mAbs did not detect the 43 kD band.

Deglycosylation of extracts of female worms by metaperiodate treatment led to a substantial loss of N11 binding in comparison with untreated samples as demonstrated by ELISA (data not shown). This result suggests that the target epitope was either a

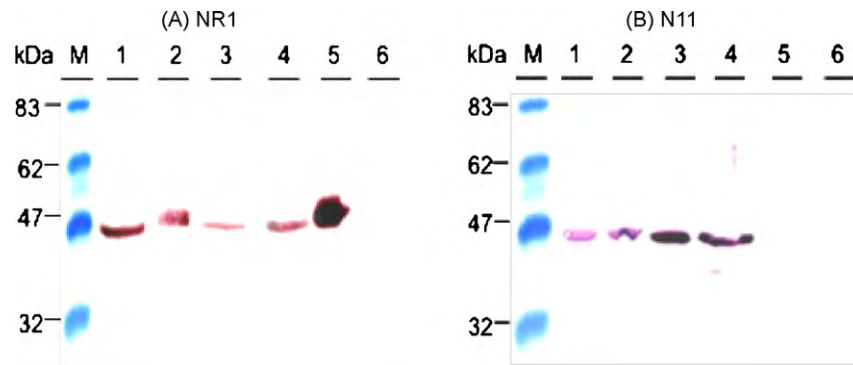


Fig. 1. Recognition in immunoblots of *A. viteae* tropomyosin by mAb NR1 (A) and mAb N11 (B): (1) extract of female worms; (2) extract of male worms; (3) mf extract; (4) L3 extract; (5) rAvTropo; (6) recombinant Pen a 1 shrimp tropomyosin.

carbohydrate moiety or influenced by carbohydrates present on worm-derived tropomyosin (data not shown). In contrast, binding efficiency of NR1 was not affected by deglycosylation of samples, suggesting the target epitope of NR1 was part of the protein backbone of tropomyosin.

3.2. The NR1-target epitope is part of tropomyosin C-terminus

The target epitope of NR1 was mapped by screening an array of overlapping 15-mer peptides representing the aa sequence of *A. viteae* tropomyosin. As shown in Fig. 2A strong binding of NR1 antibody to the peptide VDRLEDELVHEKGRY (spot 85) was observed. In comparison, intensity of spot 86 was markedly reduced due to a less pronounced interaction of peptide LEDELVHEKGRYKNI with the antibody NR1. Therefore, we determined the target epitope of

NR1 as formed by the 15-mer sequence VDRLEDELVHEKGRY at the C-terminus of the protein (Fig. 2A and D). An attempt to map the epitope in more detail by overlapping 10-mer peptides failed. We assume that the particular NR1-epitope conformation cannot be adopted by shorter peptides. It seemed as if at least two heptad repeats are necessary for recognition by the NR1 antibody. Interestingly, although the tropomyosin sequence is overall relatively conserved, comparison of *A. viteae* tropomyosin with vertebrate tropomyosins revealed major differences in this part of the protein. For example, 7 out of 15 amino acids that comprise the epitope were exchanged between porcine and *A. viteae* tropomyosin (Fig. 2D).

3.3. Helminth tropomyosins contain an invertebrate specific target epitope

To test for the specificity of NR1 and N11 we analyzed their binding by ELISA with extracts from various invertebrates and vertebrates compared with mAb TM311, directed against an epitope of chicken gizzard tropomyosin, and mAb 24-4, recognizing

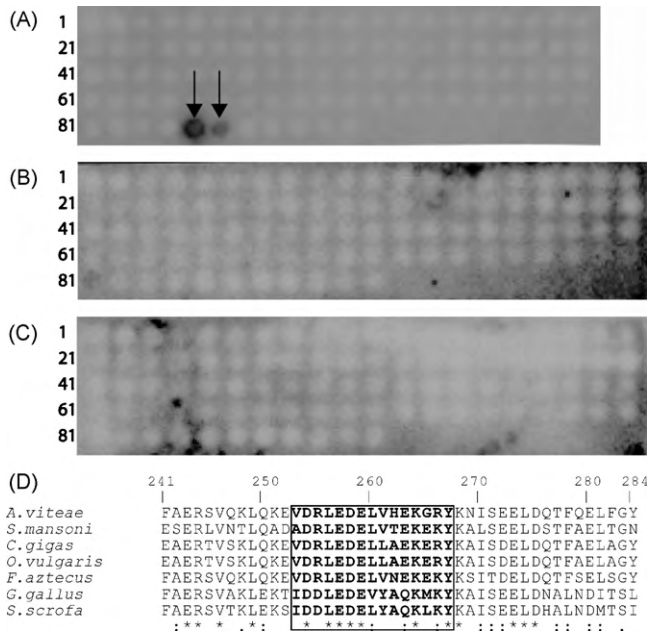


Fig. 2. Position of the target epitope of NR1. (A) A synthetic peptide array of 91 overlapping 15-mer peptides representing the complete sequence of *A. viteae* tropomyosin was applied. Overlay experiments revealed two NR1-binding peptides (spot 85 and 86, arrows). Due to the marked signal difference between spots 85 and 86 the NR1-target epitope was defined as the 15-meric sequence VDRLEDELVHEKGRY. As a control no interaction of the 24-4 (B) and TM311 (C) antibody was detected. (D) Alignment of amino acid sequences in the C-terminal region of tropomyosin of various vertebrate and invertebrate species, bearing the target epitope of NR1. Asterisks indicate identity, colons indicate conserved substitutions, dots indicate semi-conserved substitutions of amino acids. Note the relatively high homology between sequences of helminths and mollusks. Target epitope of NR1 is boxed. Numbers on left side indicate the position of 15-mer peptides.

Table 1

Recognition of PBS extracts from various invertebrates and vertebrates by mAbs NR1, N11 and control mAbs: (–) no binding, (+, ++) binding or strong binding. Listing is according to complexity of the organism, beginning with nematodes and ending with mammals.

Source of PBS extract or protein	NR1	N11	TM311	24-4
<i>A. viteae</i> female	+	+	–	–
<i>A. viteae</i> male	++	++	–	–
<i>A. viteae</i> L3	++	+	–	++
<i>A. viteae</i> mf	+	+	–	–
rAvTropo	++	–	–	–
<i>Ascaris suum</i> (nematode)	+	–	–	–
<i>Fasciola hepatica</i> (trematode)	+	–	–	–
<i>Schistosoma mansoni</i> (trematode)	++	–	–	–
<i>Hymenolepis diminuta</i> (cestode)	+	–	–	–
<i>Farfantepenaeus aztecus</i> (shrimp)	–	–	–	–
<i>Ornithodoros moubata</i> (tick)	–	–	–	–
<i>Pholcus phalangoides</i> (spider)	–	–	–	–
<i>Blaberus craniifer</i> (cockroach)	–	–	–	–
<i>Acheta domesticus</i> (house cricket)	–	–	–	–
<i>Tenebrio molitor</i> (mealworm beetle)	–	–	–	–
<i>Pachnoda sinuata</i> (flower chafer)	–	–	–	–
<i>Mytilus edulis</i> (blue mussel)	–	–	+	–
<i>Crassostrea gigas</i> (oyster)	+	–	–	–
<i>Biomphalaria glabrata</i> (snail)	–	–	++	–
<i>Octopus vulgaris</i> (octopus)	++	++	–	–
<i>Sepia pharaonis</i> (squid)	++	–	+	–
<i>Sander lucioperca</i> (perch)	–	++	++	–
<i>Theragra chalcogramma</i> (Alaska Pollock)	–	+	++	–
<i>Salmo salar</i> (salmon)	–	+	++	–
<i>Gallus gallus</i> (chicken)	–	++	++	–
<i>Passer domesticus</i> (sparrow)	–	+	++	–
<i>Sus scrofa</i> (pig)	–	+	–	–
<i>Mus musculus</i> (mouse)	–	+	+	–

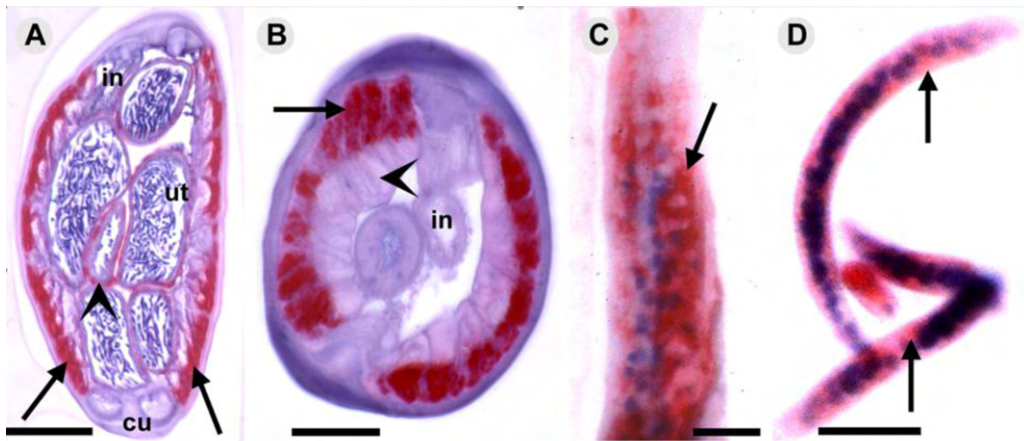


Fig. 3. Immunohistochemical analysis of histological sections of *A. viteae* showing tropomyosin labelled by mAb NR1 in the fibrillar portions of the muscles of body wall (arrow) of (A) a female worm (arrowhead: staining of uterine muscles), (B) a male worm (arrowhead: lack of staining in the afibrillar part of portion of muscles), (C) an infective larva, and (D) two mf. The afibrillar portions of the muscles, cuticle (cu), hypodermis, and the epithelia of uterus (ut) and intestine (in) are negative. Scale bar = 30 µm for (A and B) and 10 µm for (C and D).

a carbohydrate epitope of filarial chitinase. NR1 exclusively recognized tropomyosin in extracts of *A. viteae* stages, other helminths, oysters, squid and octopus, but not of other invertebrates (Table 1). The recognition of helminth and mollusk tropomyosin by NR1 coincides with a higher sequence homology between the reactive species in the region of the target epitope. N11 reacted strongly with extracts of *A. viteae* stages, with extracts of octopus and with vertebrate extracts. However, this mAb failed to bind to tropomyosin from other tested invertebrates. TM311 reacted strongest with extracts of birds and fish, but at the same time recognized extract of a snail. The control antibody 24-4 recognized only extracts of *A. viteae* L3. These data indicate that NR1 recognizes a proteinaceous tropomyosin epitope specific for platyhelminths, nematodes and some mollusks. In contrast, the binding pattern of N11 is consistent with recognition of a posttranslational modification present on tropomyosin of vertebrates and of some, but not all invertebrate species tested.

3.4. Localization of tropomyosin in helminths

NR1 and N11 were used to localize tropomyosin in various helminths using the APAAP technique. Controls omitting NR1 as primary antibody did not stain the examined helminths (not shown). The host tissues in sections with worms well stained by

NR1 were always negative, such as onchocercomas from humans, cattle, dromedaries, and red deer.

3.4.1. Localization in *A. viteae*

The fibrillar portions of the body wall muscles of female (Fig. 3A) and male (Fig. 3B) *A. viteae* and the uterus muscles (Fig. 3A) were always strongly stained by mAb NR1. In contrast, cuticle, hypodermis, epithelia of intestine, male and female genital tract, sperms, oocytes and immature embryos were not recognized (Fig. 3A and B). The muscle cells of infective larvae (Fig. 3C) and mature mf (Fig. 3D) were also always well stained. MAb N11 showed the same staining pattern of the muscles in adult worms, mf and L3 as NR1 (data not shown).

3.4.2. Localization in *O. volvulus*

To study whether the distribution of tropomyosin might vary between various strains of one filarial species, we analyzed *O. volvulus* from a larger number of human hosts ($n=47$) residing in Ghana, Liberia, Uganda or Mexico. Genetic differences between worm strains from these regions have been reported (Erttmann et al., 1987; Keddie et al., 1999). Evaluation of histological sections of onchocercomas containing living male and female worms showed that NR1 specifically stained all muscles of the filariae. The pattern of staining was very similar in worms from different origins. The

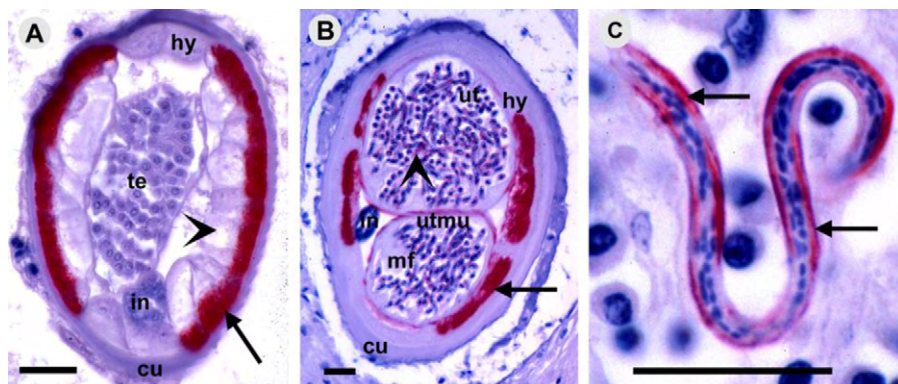


Fig. 4. Immunohistological analysis of *O. volvulus* showing tropomyosin labelled by mAb NR1 in the fibrillar portions of all muscles (arrows) whereas the afibrillar portions of the muscles (arrowhead in A) and other tissues are negative. (A) Male filaria with positive staining in body wall muscles. (B) Coiled and immature stretched mf within the uterus of a female worm are still negative, while a mature mf is positive (arrowhead). (C) A mf in the human tissue of the nodule shows stained muscles (arrow) in contrast to the negative central cells: cuticle (cu); hypodermis (hy); intestine (in); microfilaria (mf); testis (te); uterus (ut); uterus muscles (utmu). Scale bar = 30 µm.

Table 2
Helminths with muscles strongly stained by mAb NR1.

Helminth species	Host	Origin
<i>Acanthocheilonema viteae</i>	<i>Meriones unguiculatus</i>	Laboratory
<i>Onchocerca volvulus</i>	Human patients	Ghana, Liberia, Uganda, Mexico
<i>Onchocerca fasciata</i>	Dromedary	Saudi Arabia
<i>Onchocerca gibsoni</i>	Cattle	Australia
<i>Onchocerca gutturosa</i>	Cattle	Uganda
<i>Onchocerca flexuosa</i>	<i>Cervus elaphus</i>	Germany
<i>Onchocerca jakutensis</i>	<i>Cervus elaphus</i>	Germany
<i>Wuchereria bancrofti</i>	Human patient	Ghana
<i>Brugia malayi</i>	<i>Mastomys coucha</i>	Laboratory
<i>Loa loa</i>	Human patients and human strain in drills	Cameroon
<i>Parastrongylus costaricensis</i>	Rodent	Laboratory
<i>Ascaris suum</i>	Domestic pig	Germany
<i>Anisakis</i> sp.	Herring	Germany
<i>Schistosoma mansoni</i>	White mouse	Laboratory

staining was limited to the fibrillar zone of the muscles, whereas the afibrillar zone was negative (Fig. 4A). This distribution was particularly conspicuous in the well developed body wall muscles of the male worms and in the uterus muscles. In males, the muscles involved in the movements of the spicules were also strongly stained (not shown). In females, the muscle cells of vagina (not shown), uterus (Fig. 4B), oviduct and pharynx were intensively immunostained (data not shown). NR1 did not label the cuticle, the intestinal epithelium and the epithelia of the male and female genital tracts or any stages of sperms (Fig. 4A and B). The oocytes (not shown) and all younger embryos were negative (Fig. 4B). The first stained stages were the stretched mf within the uterus, which had left the eggshell (Fig. 4B). NR1 strongly stained the muscles of mature mf in the human tissue of onchocercomas and in the skin, while the central region of the mf was negative (Fig. 4C). No staining was seen in the pseudocoeloma cavity (Fig. 4A and B) or in the cavities of any organs (Fig. 4A and B), suggesting that the protein is not secreted.

3.4.3. Localization in other worms

Sections of a small number of other worm species were studied using mAb NR1. Strong staining of the body wall muscles was observed in all examined adult nematodes (Table 2, Fig. 5A and B), in a second or third stage larva of *Anisakis* sp. (Fig. 5C) and in infective larvae of *Wuchereria bancrofti* (not shown). Muscles of the male trematode *Schistosoma mansoni* were weakly, but specifically

stained (Fig. 5D). In contrast, female *S. mansoni* were not stained (Fig. 5D). The muscles of the leech *Hirudo medicinalis* were negative as well as the thorax muscles and other tissues of the mosquito *Anopheles gambiae* and the blackfly *Simulium yahense* (not shown). These findings are in accordance with the results presented in Table 1.

4. Discussion

The presented data describe tropomyosin as a constituent of various helminth species and illustrate its distribution within some worm species by immunolocalization. Nematode tropomyosin is considered as an allergen on the basis of findings that humans were shown to have IgE responses against tropomyosin of *Anisakis* (Asturias et al., 2000; Audicana and Kennedy, 2008) and *A. viteae* infected birds develop IgE antibodies against tropomyosin (Hartmann et al., 1997). Moreover, not only natural infection but also immunization with recombinant *A. viteae* tropomyosin induced high levels of IgE that can sensitise rat basophile leukaemia cells to degranulate in the presence of *A. viteae* tropomyosin (Hartmann et al., 2006). Tropomyosin was recently described as the major allergen of *Ascaris*, based on its cross-reactivity with mite tropomyosin (Acevedo et al., 2009).

Our experiments with mAb NR1 suggest that it recognizes an epitope of helminth tropomyosin shared with marine mollusks (octopus, squid and oyster), as shown by comparison of muscle extracts of various organisms and by immunoblotting. This is another example of common B cell epitopes of invertebrate tropomyosins. Antigen commonalities of invertebrate tropomyosins have been described earlier on the basis of serological cross-reactions for example between tropomyosin of shrimps and cockroaches, fruit fly, house dust mites and silverfish (reviewed in Martinez et al., 1997; Lopata and Lehrer, 2009). So far none of the epitopes cross-reacting between tropomyosins of various species has been defined with mAbs. Interestingly, NR1 did not recognize tropomyosin of crustaceans, acarids and insects. This limitation of cross-reactivity to a few taxa of invertebrates is consistent with the described pattern of immunogenicity of tropomyosins (Lopata and Lehrer, 2009; Arruda and Santos, 2005; Jeong et al., 2006). Cross-reactions between tropomyosins of invertebrate species are of potential medical relevance, as for example an individual with nematode infection might be misdiagnosed to be allergic against seafood, as was reported in cases of *Anisakis* infection giving false positive food allergy results (Guarneri et al., 2007). Restimulation of IgE responses originally developed by contact with helminth tropomyosin might also lead to allergic reactions against squid or

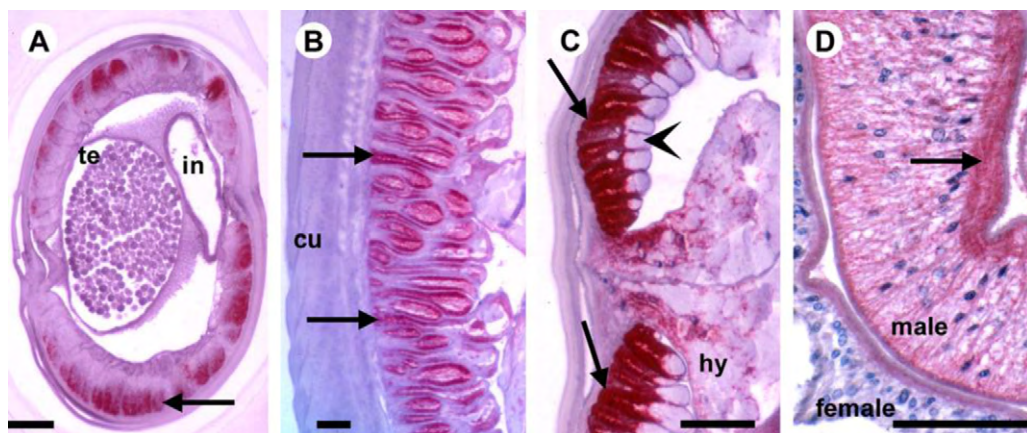


Fig. 5. Immunohistochemistry of several helminths showing tropomyosin labelling by mAb NR1 in the muscles (arrows). (A) Male *Loa loa* filaria. (B) Female intestinal nematode *Ascaris suum*. (C) Second or third stage larva of the intestinal nematode *Anisakis* sp. with negative afibrillar portions of the muscles (arrowhead). (D) The male trematode *S. mansoni* shows well stained subtegumental muscles, the female not: cuticle (cu); hypodermis (hy); intestine (in); testis (te). Scale bar = 30 µm.

octopus. We did not test whether the target epitope of the IgG1 mAb NR1 is recognized by IgE antibodies. This is, however, possible, as the class of an antibody with a given antigen-recognizing specificity can switch as a consequence of cytokine-induced signaling. Further studies have to reveal whether the NR1-target epitope is of clinical significance, which could be the case if it were recognized by IgE antibodies of seafood allergic patients. An invertebrate specific monoclonal antibody like NR1 can be used to discern from host tissues parasites that are otherwise difficult to detect (see, e.g. Fig. 4C). It is likely that besides the NR1-target epitope, other tropomyosin epitopes are shared between invertebrates, which would cause cross-reactions between nematodes and shrimps (Jenkins et al., 1998), house dust mites (Jeong et al., 2006) and cockroaches (Santos et al., 2008). The recognition pattern of the second mAb, N11, demonstrates that invertebrate tropomyosins at the same time share epitopes with vertebrate tropomyosins.

Within the studied filarial nematodes NR1 epitope was present in the fibrillar part of muscle cells. It stained muscle cells of various organs of adult worms, for example body wall, uterus wall, esophagus and vagina. The protein occurred in all stages of the rodent filarial nematode *A. viteae*. As our strain of *A. viteae* has been kept in the laboratory for several decades and is likely to be genetically homogenous, we also studied the distribution of tropomyosin in the human pathogenic filaria *O. volvulus* from different geographic origins. This analysis did not reveal strain specific differences regarding the pattern of distribution of tropomyosin. Also, further *Onchocerca* species and other filariids like *Loa loa*, *Brugia malayi* and *W. bancrofti* showed a similar pattern of protein distribution. Thus, it can be assumed that filarial nematodes in general contain substantial amounts of tropomyosin in their muscles. This presence of tropomyosin also pertains to other helminths, as revealed by our ELISA studies and our immunostaining analysis of other parasites.

As we have no clear indication for secretion of tropomyosin by filarial nematodes, we assume that hosts get into contact with this protein when mf or adult worms are killed by immune attack or when dead nematode stages are decomposed by phagocytes. As filarial nematodes produce large amounts of microfilariae, these larvae are probably a major source of allergens, which continuously challenge the host immune system. Together with other nematode allergens (Kennedy, 2000; Shaw et al., 2003; Orton et al., 2007), this exposure should theoretically lead to severe allergic responses. The inflammation triggered by such immune responses would probably endanger the parasites, exposing them to various types of highly efficient immune cells and their aggressive effector molecules. Yet, several studies on the effect of anthelmintic treatment of humans revealed that worm infections can reduce allergic skin reactivity (Lynch et al., 1998; van den Biggelaar et al., 2004; Flohr et al., 2010). In the same line, data from animal models demonstrate that worm infections can reduce allergic disease (see Harnett and Harnett, 2008). It has been clearly demonstrated that certain nematode proteins are capable of down-regulating allergic and inflammatory responses (Melendez et al., 2007; Schnoeller et al., 2008; Park et al., 2009). However, it is worthwhile to study whether helminth allergens per se have certain qualities that would interfere with allergic effector mechanisms. An immunomodulating effect of helminth tropomyosin would not be surprising, given the specificity of adaptations acquired by parasites during co-evolution with their hosts. Highly specific reagents like NR1 could be helpful to dissect this aspect of parasite host interplay.

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