

Research Note

A house dust mite allergen homologue from poultry red mite *Dermanyssus gallinae* (De Geer)*

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SUMMARY

Tropomyosin is an allergenic, actin-binding protein and a proposed vaccine candidate from several species of parasite. Tropomyosin cDNA, obtained by polymerase chain reaction (PCR) amplification from Dermanyssus gallinae RNA, encoded a predicted protein with 89% and 88% identity to tropomyosins from the ticks Boophilus microplus and Haemaphysalis longicornis, respectively, and 85% identity to the house dust mite (HDM) tropomyosin Der p 10. Mouse antibodies raised against HDM tropomyosin reacted with a band of 38 kDa on Western blots of D. gallinae extract, consistent with the molecular masses of acarine tropomyosins and the putative product of the cDNA encoding D. gallinae tropomyosin. When the same preparation of D. gallinae proteins was used in Western blots with serum from infested hens, the IgY component of the serum bound to a number of mite proteins, but not to tropomyosin, indicating that hens are not directly exposed to this allergen during a natural infestation. Immunolocalization of tropomyosin in mites indicated a ubiquitous distribution of the molecule in mite tissues. Immunolocalization and Western blotting also indicated that poultry red mites ingest host IgY.

Keywords *Der p 10, Dermanyssus gallinae, mite allergen, poultry red mite, tropomyosin*

RESEARCH NOTE

Dermanyssus gallinae, the poultry red mite, is a cosmopolitan parasite of fowl, cage birds and wild birds. In laying hen production systems, *D. gallinae* is an important ectoparasite and infestation with these mites is a health and welfare issue for hens world-wide [e.g. see (1)], capable of causing major reductions in bird performance (2), the development of a potentially fatal anaemia (3,4) and an increased incidence of aggressive pecking and cannibalism (4). Poultry red mites have also been implicated as intermediate hosts for a number of important poultry diseases (1,5). In addition to the effects of the parasites on the birds which they infest, *D. gallinae* has also been implicated in the production of a pruritic dermatitis in poultry workers when they come into contact with the mites in poultry houses (6). Although there are a number of chemical pesticides available for mite control (1), there is evidence that the poultry red mite in a number of EU countries has developed heritable resistance to acaricides (7,8). In addition, residues of acaricides, such as propoxur, in eggs from laying hens have been detected at more than six times the permitted levels in the EU (9). There is therefore a significant and growing requirement to develop alternative methods of control, such as the use of a vaccine, against poultry red mite.

The group 10 house dust mite allergen, tropomyosin, is an allergenic, actin-binding protein which has been shown to react with specific IgE in the sera of humans allergic to house dust mite at a wide range of frequencies, from 5·6% (10) to 81% (11); a variability that may, at least in part, result from the source of sera and of prior sensitization of some individuals to cross-reacting tropomyosins from other species (12). Tropomyosin has also been shown, by Western blotting using anti-HDM tropomyosin sera, to be present in extracts of the sheep scab mite *Psoroptes ovis*, and to provoke the production of IgE and IgG responses in the sheep host

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during infestation (13). In addition, tropomyosin has shown some potential as a vaccine candidate against species of nematode. For example, immunization of jirds with a tropomyosin-like molecule from the nematode *Acanthocheilonema viteae* reduced adult worm burden by > 60% and circulating microfilariae by up to 93% (14).

The research described here aimed to characterize tropomyosin of the poultry red mite, to investigate its immunogenicity and tissue distribution, and to determine its potential role as a natural antigen in infested poultry houses. Knowledge of these factors is prerequisite to examine its potential as a mediator of allergic disease in the host and in poultry workers and/or its potential as a vaccine candidate.

Mites were collected from a naturally infested poultry house, snap-frozen in liquid nitrogen and stored at -80°C prior to RNA extraction. Total RNA was extracted from mixed sex adult *D. gallinae* under liquid nitrogen by homogenization in TriPure™ reagent (Roche) according to the manufacturer's protocol and stored at -80°C . Degenerate primers were designed based on conserved regions of the published sequences of acarine tropomyosins. The sequences of these primers were: Derg10F; 5'ATGGA (C/G)GCCATCAA (A/G)AA (A/G/T)AA (A/G)ATGCAGGC3' and Derg10R; 5'CC (A/C/G)GTGAG (C/T)TCGGA (A/G)AA (A/G/T)GT (C/T)TGGTCCA3'. Reverse transcription and cDNA amplification were performed using 1 µg of the RNA, each degenerate primer (final concentration 0.4 µM) and the SuperScript™ One-Step RT-PCR System (Invitrogen) in accordance with the manufacturer's instructions. Primers Derg10F and Derg10R were then used in conjunction with Universal Primer Mix (UPM, BD Biosciences) to amplify the tropomyosin coding region by rapid amplification of cDNA ends (RACE) using the SMART™ RACE cDNA Amplification Kit (BD Biosciences), according to the manufacturer's protocol. The product obtained by PCR amplification using Derg10 and UPM primers contained an open reading frame (ORF) of 852 bp (EMBL accession number AM167555) which encoded a predicted protein with 89% and 88% identity to tropomyosins from the ticks *Boophilus microplus* (NCBI accession number AAD17324) and *Haemaphysalis longicornis* (NCBI accession number AAN05633), respectively, and 85% identity to the HDM tropomyosin Der p 10 (NCBI accession number AAB69424). The predicted protein contained 284 amino acids residues, characteristic of a muscle isoform rather than the smaller non-muscle isoforms of the molecule, and the consensus C-terminal signature sequence for tropomyosins [LK (EAD)AExRA (ET)] was located at L₂₃₂ to E₂₄₀ in the predicted sequence. The heptad repeat of amino acids (*a-b-c-d-e-f-g*, where *a* and *d* are generally apolar residues, e.g. L₂₃₂ and A₂₃₅ at positions *a* and *d* in the signature sequence) which characterizes coiled-coil molecules (15) was found in

40 repetitive units across the length of the predicted protein, and more than 30% of the residues at the '*d*' position of the heptad were filled by alanine in the predicted *D. gallinae* tropomyosin. As with the tropomyosins from *B. microplus* and *H. longicornis*, the lysine residue in the signature sequence at K₂₃₃ is replaced by glutamine. Phylogenetic analysis of predicted tropomyosin proteins from *D. gallinae* and other selected organisms revealed strong bootstrap support, using the neighbour-joining algorithm, for the grouping of the *D. gallinae* tropomyosin with the sequences from ticks, and in a distinct group from the sequences of the Astigmatid mite species. This reflects the phylogenetic status of *D. gallinae* which, as a Mesostigmatid mite, belongs to the superorder Anactinotrichida (order Parasitiformes) with ticks (suborder Ixodida), whereas the Astigmatid house dust mites and sheep scab mite are classified in the superorder Actinotrichida (16). Nevertheless, the high levels of sequence conservation between HDM and *D. gallinae* tropomyosin allowed mouse antibodies, raised against HDM tropomyosin (kindly donated by Prof K.Y. Chua, National University of Singapore), to react specifically with a band of 38 kDa on Western blots of soluble extracts of *D. gallinae* (data not shown), consistent with the molecular masses of known acarine tropomyosins (13) and with the putative product of the cDNA described above.

When the same soluble preparation of *D. gallinae* proteins was probed with sera from infested hens, the IgY component bound to a number of mite proteins, particularly in the higher molecular weight range (60–150 kDa), but not to tropomyosin. Western blotting also indicated that *D. gallinae* feeding on hens ingest the IgY component of serum (data not shown).

When mouse antibodies raised against HDM tropomyosin were used to probe sections of *D. gallinae*, specific staining was observed throughout the sections in mite tissues, reflecting the ubiquitous nature of these proteins and their biological roles (Figure 1a). The immunoreactivity observed throughout the mid and posterior regions of the mite is related to the numerous, small muscle bundles associated with the digestive and reproductive systems. No specific immunolabelling was observed in the control material (Figure 1b).

Despite the ubiquitous nature of tropomyosin in the internal organs of the mite, serum from hens housed in the infested premises did not contain IgY which reacted with poultry red mite tropomyosin. Tropomyosin is a strongly antigenic protein which has previously been shown to provoke the production of antibody responses (IgE in particular) in sheep during infestation with the sheep scab mite *P. ovis* (13). Avian IgY is believed to be the evolutionary precursor of mammalian antibody classes IgG and IgE despite fundamental structural differences between the molecules (17), however, an important difference in the feeding and living habits of *D. gallinae* and *P. ovis* may explain the

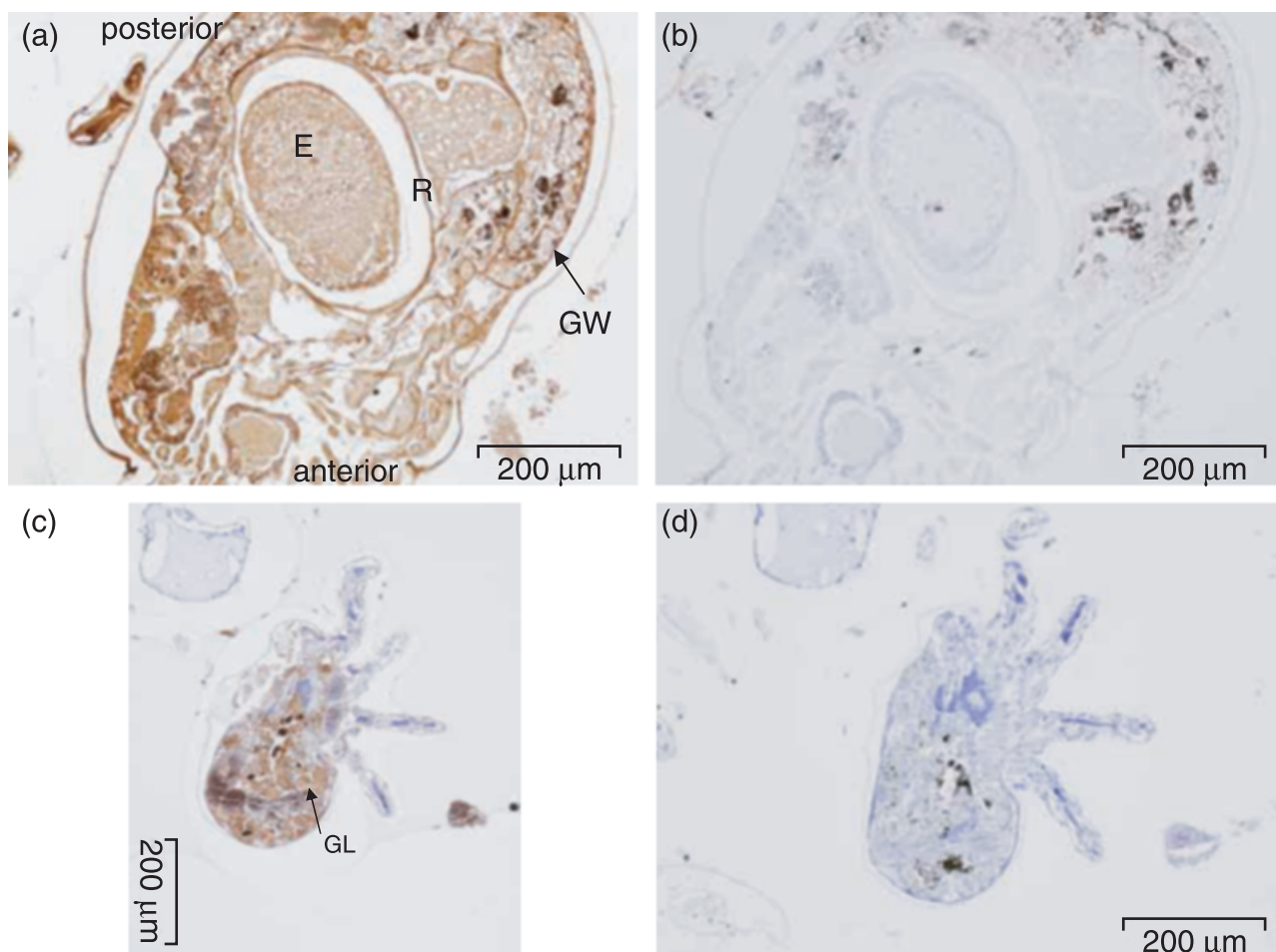


Figure 1 Immunolocalization of tropomyosin and hen IgY in sections of *Dermanyssus gallinae*. Live mites were fixed in Carnoy's fixative (60% ethanol, 30% chloroform, 10% acetic acid) and stored at 4°C until ready for processing. Following removal of fixative, mites were placed in 1% molten agarose and left to solidify before processing into paraffin wax. 5 µm sections were dried at 40°C, dewaxed in xylene and rehydrated. Sections were treated to remove endogenous peroxidase (EnVision Kit, DAKO) and blocked in 25% normal goat serum in Tris buffered saline (TBS; 25 mM Tris, 150 mM NaCl, 3 mM KCl) for 30 min before immunolabelling using mouse polyclonal anti-HDM tropomyosin (1 : 1000) or serum raised in rabbit against chicken IgY (HRP conjugate 1 : 100, Sigma) at 4°C overnight. The EnVision + System-HRP (DAB) (DAKO) was used, according to manufacturer's instructions, for detection of the mouse antibodies. Negative controls were prepared by omitting the primary antibodies. Sections were washed in water, counterstained in haematoxylin for a few seconds, washed in water, dehydrated, cleared and mounted in a resin based mountant. Panel (a), staining with mouse anti-HDM tropomyosin. Annotation: E, egg; R, reproductive tract, GW, gut wall. Panel (b), control (no primary antibody). Panel (c), staining with rabbit anti-chicken IgY. Annotation: GL, gut lumen. Panel (d), control (no rabbit anti-chicken IgY).

differences in host antibody responses to the tropomyosins of the two mite species: *P. ovis* completes its entire life cycle on the host and for much of its life the mite exists within, or at the edge of, an exudative lesion on the surface of the host. Sheep scab mite antigens from excretions/secretions and those resulting from the posthumous degradation of the mites are therefore in constant contact with host serum. In contrast, poultry red mite nymphs and adults visit the host periodically to feed before returning to cracks and crevices in the structures or furniture in poultry houses to digest the blood-meal and develop or lay eggs. The mites are therefore only

in direct contact with the host for short periods of time and, if tropomyosin was to be transferred to the host, it could only be via the saliva. Paramyosin, which is a structural protein in invertebrate muscles and has a similar tissue distribution to tropomyosin in *P. ovis* (Nisbet *et al.* unpublished), is distributed throughout the internal organs of the tick *B. microplus* (18). However, in spite of the presence of the molecule in salivary glands, it was absent from saliva harvested from ticks and therefore not exposed to the host during tick infestation. It seems possible that the same situation could occur for *D. gallinae* tropomyosin.

The presence of tick paramyosin in the gut, its potential exposure to ingested host blood and the demonstrated ability to interact with components of the host's immune system has led to the suggestion that it may represent an important new 'concealed' antigen for vaccination studies (18). In addition, recombinant versions of the muscle-associated protein P27/30, a troponin I-like molecule, have recently been used successfully to produce a protective response in rabbits and mice against infestation with the tick *Haemaphysalis longicornis* (19,20). Troponin I is a member of a complex containing troponin C which binds Ca^{2+} , troponin I which binds actin (thus inhibiting actin-myosin interactions), and troponin T which binds to tropomyosin (21). These studies with ticks have therefore demonstrated the potential of using muscle-associated proteins in vaccines against ectoparasites.

When used in vaccination trials in other species of parasite, tropomyosin has shown mixed levels of success. For example, immunization of jirds with a 41 kDa tropomyosin-like protein purified from the filarial nematode *Acanthocheilonema viteae* reduced adult worm burden by > 60% and circulating microfilariae by up to 93% (14). However DNA immunization of mice with plasmid expressing the *Onchocerca volvulus* antigen, Ov-TMY-1 (tropomyosin) induced strong Th1/Th2 IgG isotype profiles but failed to protect against infection on challenge (22).

We have demonstrated that tropomyosin is present in the gut of *D. gallinae* and that the mite ingests IgY from its host during feeding (Figure 1c,d). The demonstration that poultry red mites ingest hen IgY is perhaps unsurprising as the mites ingest whole blood from the host, giving them their characteristic red colour. However, it is important to demonstrate the presence of this host molecule in the gut of the mite if the possibility of vaccination is to be explored (23).

In conclusion, we have demonstrated the presence of a homologue of the house dust mite allergen Der p 10 in the poultry red mite *D. gallinae*. This homologue is a tropomyosin which is distributed throughout the tissues of the mite but does not act as a natural antigen during infestation of the host. Based on previous studies with tropomyosins of parasites, the molecule may represent a candidate for the 'concealed antigen' approach to vaccination.

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