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SHORT COMMUNICATION

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A 41-kDa antigen of the rodent filaria *Acanthocheilonema viteae* with homologies to tropomyosin induces host-protective immune responses

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Abstract A purified 41-kDa protein of the rodent filaria *Acanthocheilonema viteae* was shown to protect jirds against a challenge infection. Subcutaneous immunization with the protein reduced the number of adult worms by up to 65% and the number of circulating microfilariae declined by up to 93% in these animals. The protein is located in the muscle tissues of adult worms and was identified as tropomyosin by N-terminal sequencing of the purified protein.

Abbreviations *PBS* Phosphate-buffered saline, *FITC* fluorescein isothiocyanate, *IFAT* indirect immune fluorescence antibody test, *L3* infective-stage larvae, *Mf* microfilariae

Although more than 100 million people throughout the tropics are infected with the filarial parasites *Onchocerca volvulus*, *Brugia malayi*, and *Wuchereria bancrofti* (WHO 1992), few host-protective parasite molecules have been identified (Kazura et al. 1990; Li et al. 1993; Raghavan et al. 1994). In animal models, immunization with irradiation-attenuated L3 has been demonstrated to be a potent method to induce protective immunity (Philipp et al. 1988). As most human filariae are strictly primate-specific, which limits in vivo studies on biological functions of parasite molecules, we studied the infection of the jird (*Meriones unguiculatus*) using *Acanthocheilonema viteae* as a model. This host-parasite system is considered to be close to the natural life cycle since jirds are related to rodent species, which have been found to be naturally infected (Worms et al. 1961). Vaccination of jirds with irradiated L3 of *A. viteae* induces approximately 90% resistance (Lucius et al. 1991) and demonstrates the occurrence of protective immunity.

In this paper we describe an *A. viteae* antigen that is recognized by a monoclonal antibody (mAb 29–16) raised against irradiated L3. For the production of this mAb (IgM, kappa light chain), 6-week-old BALB/c mice were immunized twice at 2-week intervals by subcutaneous injection of 50 irradiated L3 of *A. viteae*. At 5 days prior to the fusion, the animals were boosted with a further dose of irradiated L3 given intraperitoneally. Spleen cells of these animals were fused with BALB/c myeloma X63/Ag8 cells and hybridomas were selected according to standard methods (Galfre and Milstein 1981). Immunoblot studies revealed that mAb 29–16 detected a 41-kDa band in PBS-soluble antigen extracts of *A. viteae* L3, microfilariae (Mf), and adult male and female worms. We used mAb 29–16 to purify the 41-kDa band from the PBS-soluble extract of adult female worms by immunoaffinity chromatography. As only small amounts of the 41-kDa antigen could be purified by this method, the 41-kDa band was excised out of a sodium dodecyl sulfate (SDS)-polyacrylamide gel and the protein was electroeluted.

The affinity-purified 41-kDa protein and the gel-eluted 41-kDa protein were used to immunize jirds. In brief, 2 μg of the protein emulsified in the mild adjuvant STP (Byars and Allison 1987) was applied three times at 2-week intervals by subcutaneous injection under the skin of the flanks as previously described elsewhere (Lucius et al. 1991). Challenge doses of 80 fully motile L3 were injected under the skin of the neck. At 12 weeks p.i. the animals were dissected and the worm burdens and Mf densities were determined. Immunization with 2 μg of affinity-purified 41-kDa protein resulted in a mean reduction of 25.4% of the worm burdens, and

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Table 1 Effect of immunization with the 41-kDa protein of *Acanthocheilonema viteae* on challenge infection with 80 L3 of *A. viteae*

	Experiment 1			Experiment 2	
	Affinity-purified 41-kDa	Gel-eluted 41-kDa	Challenge control	Gel-eluted 41-kDa	Challenge control
Immunization	3 × 2 μg	3 × 2 μg	3 × PBS	3 × 25 μg	3 × PBS
Challenge infection	80 L3	80 L3	80 L3	80 L3	80 L3
Recovery of adult worms at dissection (*n*, mean ± SD)	17.4 ± 10.7	17.7 ± 10.7	23.8 ± 8.0	10.6 ± 8.2*	29.7 ± 8.1
Female worm length (cm, mean ± SD)	4.7 ± 0.4	4.5 ± 0.5	4.5 ± 0.3	3.4 ± 0.3*	4.8 ± 0.5
Male worm length (cm, mean ± SD)	2.2 ± 0.2	2.2 ± 0.2	2.3 ± 0.2	2.1 ± 0.3	2.4 ± 0.4
Mf density at dissection (cm, mean ± SD)	17.8 ± 11.5	10.7 ± 2.9	19.1 ± 14.3	2.4 ± 2.9*	33.7 ± 31.2
Surviving animals (*n*)	5/5	4/5	7/10	5/5	8/11
% Protection according to worm burden	25.4	24.1	–	64.4	–

*$P < 0.05$ according to the Wilcoxon rank test

immunization with a total dose of 6 μg of the gel-eluted 41-kDa protein reduced the worm burdens by 24.1 % in comparison with the challenge controls (Table 1). In a second experiment we used 25 μg of the gel-eluted 41-kDa protein for the immunization of jirds and achieved a significant reduction of 64.6 % ($P < 0.05$) of the worm burden and a significant reduction in the circulating Mf by 92.9 % ($P < 0.05$) in comparison with the challenge control animals (Table 1).

To characterize the 41-kDa molecule further, we determined the N-terminal sequence of the protein. For this purpose, gel-eluted 41-kDa protein was blotted onto a polyvinylidine difluoride membrane (Immobilon, Millipore, Bedford, Mass.) and digested in situ with trypsin (Boehringer, Mannheim; Aebersold et al. 1987). Three resulting fragments were purified by high-performance liquid chromatography (HPLC) and their N-termini were sequenced using an ABI 477 protein sequencer (Matsudaira 1987). A data-base search of the EMBL and GenBank data bases using the HUSAR network service of the German Cancer Research Center, Heidelberg, based on the program package of GCG Inc. (Madison, Wis.; Devereux et al. 1984) revealed striking homologies for each peptide with tropomyosins of parasitic nematodes. Figure 1 shows an amino acid comparison between *O. volvulus* and *Trichostrongylus colubriformis* tropomyosins and the three peptides obtained from the 41-kDa protein of *A. viteae*. An amino acid identity of 60 %, 70 %, and 88 %, respectively, was determined between the three *A. viteae* peptides and the nematode tropomyosins mentioned above.

The identification of the 41-kDa protein as tropomyosin was supported by localization studies with mAb 29–16 in an IFAT. Cryostat sections of 5-μm thickness were prepared from adult *A. viteae* male and female worms wrapped into mouse muscle tissue. The sections

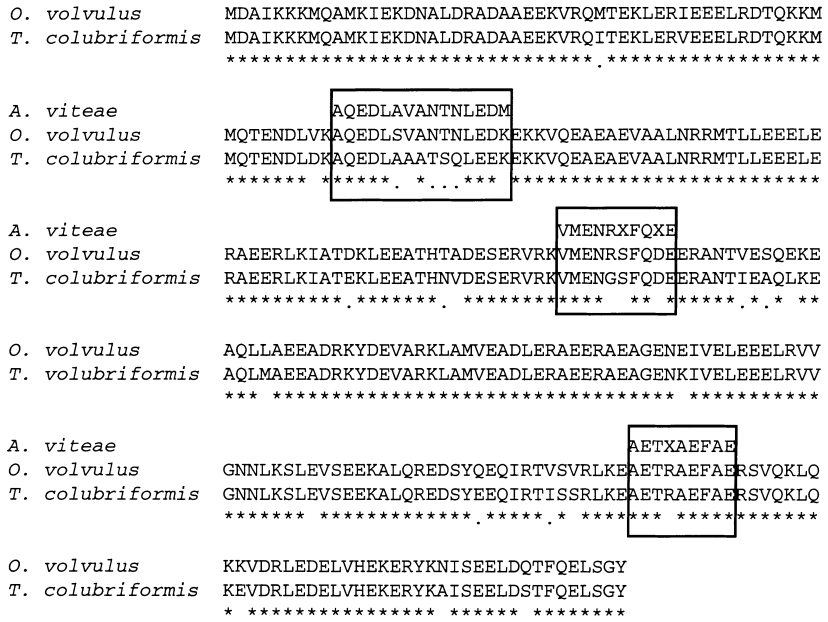

Fig. 1 Amino acid comparison of peptide sequences of *Onchocerca volvulus* tropomyosin, *Trichostrongylus colubriformis* tropomyosin, and the 41-kDa protein of *Acanthocheilonema viteae*. Identical amino acids are marked by an *asterisk*; similar amino acids are marked by a *point*. The three peptides obtained by N-terminal sequencing of the gel-eluted and digested 41-kDa protein of *A. viteae* are enclosed in *boxes*

were fixed on glass slides with methanol and were reacted sequentially with undiluted hybridoma supernatants of mAb 29–16 or an isotype-matched control mAb and FITC-conjugated anti-mouse IgG + IgM secondary antibodies. The target epitope of mAb 29–16 was shown to be associated with nematode muscle cells, as they showed a strong fluorescence, whereas the control IgM antibody did not detect muscle cells (Fig. 2). These findings are in accordance with the described localization of tropomyosin as a ubiquitous component of muscle and cytoskeleton (Anderson 1989).

Interestingly, tropomyosin of another parasitic nematode of sheep, *T. colubriformis*, has also been found to induce host-protective immune responses. Immunization with 50 μg of a 41-kDa molecule of L3 of *T. colubriformis* has been described to induce a level of protection ranging between 43% and 51% against a challenge infection in guinea pigs (O'Donnell et al. 1989). This detergent-solubilized 41-kDa extract of *T. colubriformis* was subsequently identified and characterized as tropomyosin (Frenkel et al. 1989).

As tropomyosin regulates the calcium-sensitive interaction of actin and myosin in striated muscles in association with troponins, it has an essential function for the motility of the nematodes. In the absence of Ca^{2+} the presence of troponin and tropomyosin on the actin filaments inhibits adenosine triphosphatase activity by blocking the interaction of the myosin heads with actin. Although tropomyosin is not exposed on the cuticula of intact nematodes, the internal location does not seem to exclude its function as an immunologically relevant component. In parallel, another protein of invertebrate muscle, paramyosin, has been demonstrated to be a protective parasite antigen. In murine schistosomiasis, immunization with paramyosin has induced protective immunity that is assumed to be T-cell-dependent (Pearce et al. 1988). Additionally, paramyosin has been found in filarial infections to be preferentially recognized by sera from jirds vaccinated with irradiated *Brugia malayi* infective larvae (Li et al. 1991), and mice immunized with native paramyosin have shown enhanced clearance of microfilariae (Nanduri and Kazura 1989; Li et al. 1993).

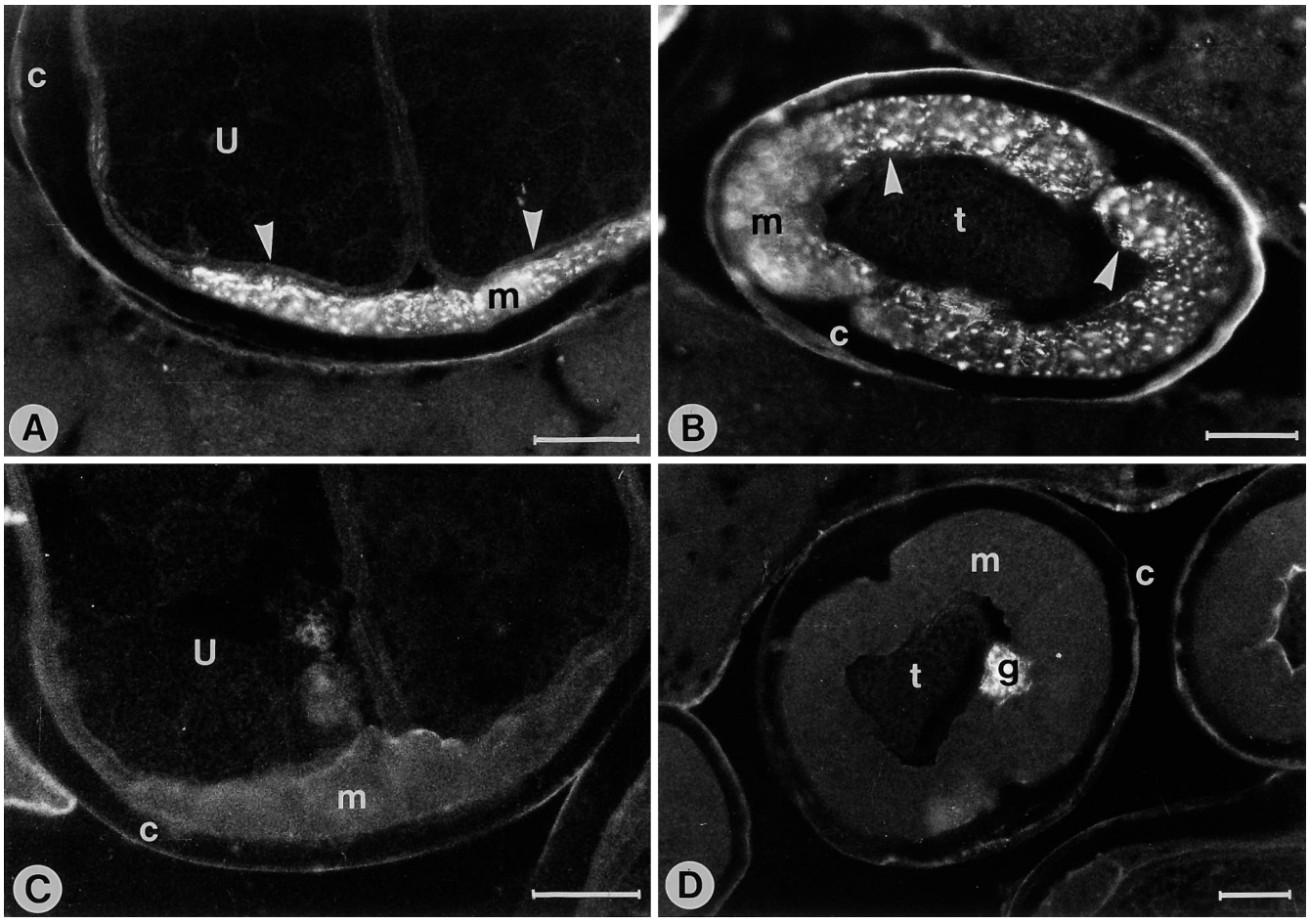

Fig. 2A–D IFAT with cryostat sections of female and male worms of *A. viteae*. **A, B** Reaction obtained with mAb 29–16 on **A** female and **B** male worm sections, respectively. Note the strong fluorescence of the muscle indicated by *arrow-heads*. **C, D** Control reaction observed with an irrelevant IgM mAb on **C** female and **D** male worm sections, respectively, using an isotype-matched control mAb. The fluorescence of the gut is due to nonspecific binding of mAbs (*c* Cuticle, *m* muscle, *U* uterus, *t* testes, *g* gut). *Bars* 50 μm

Molecular cloning of the *A. viteae* cDNA of tropomyosin and production of recombinant antigens will allow us to extend our studies of the host protective role and immunological properties of tropomyosin.

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