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A nematode allergen elicits protection against challenge infection under specific conditions

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Abstract

We describe tropomyosin of the filarial nematode *Acanthocheilonema viteae* as an allergen and study its protective potential in the natural rodent host *Meriones unguiculatus* (jird). Jirds immunized with recombinant *E. coli*-expressed *A. viteae* tropomyosin emusified in alum were not protected, while immunization with recombinant *A. viteae* tropomyosin or with protein purified from worms together with the adjuvant STP led to reduction of adult worm burdens by 30%. Vaccination with cDNA induced protection of about 30%, while application of cDNA together with aluminium phosphate increased the protection to >40%. Our data suggest that vaccination with tropomyosin under Th1 conditions, which are untypical for nematode infections, induces protection via an antibody independent effector mechanism.

Keywords: Nematode; Tropomyosin; Allergen

1. Introduction

Filarial nematodes are endoparasites that dwell subcutenous tissues or lymphatics of their hosts. Various studies in animal models of filariasis have shown that substantial protective immune responses against these parasitic nematodes are reliably induced by immunization with attenuated infective stage three larvae (L3) or by immunization with ES antigens of L3 [1,2]. In contrast, vaccination with *E. coli*-expressed proteins of filariae has met with difficulties, because the level of protection observed was generally much lower than with irradiated larvae [3,4] and the protective effect of recombinant proteins showed high variability between experiments [5]. Several factors could account for the generally low performance of vaccines utilizing single *E. coli*-expressed proteins as compared to living worms or complex ES-proteins. It is possible that not a single protein, but several antigens in concert might be necessary to induce efficient protective effector mechanisms. In addition, *E. coli* derived proteins might be unsuitable because they lack critical epitopes owing to improper folding of proteins or absence of post-translational modifications. For example, glycan epitopes were suggested to be targets of protective antibody responses against intestinal nematodes [6,7]. Furthermore, it is conceivable that a particular immunization scheme is necessary to induce relevant immune effector mechanisms.

The type and efficiency of an immune response against an immunizing protein among other factors depend critically on the adjuvant used, the route of immunization and the mode of antigen delivery [8]. The adjuvant aluminum hydroxide (alum) promotes Th2 responses and the production of antibodies, among others high levels of IgE [9] and alum is generally not considered efficient in raising cell-mediated immune responses [10]. In contrast, the adjuvant STP (a mixture of Squalane, Tween and the synthetic Coblock polymer "Pleuronic") predominantly promotes Th1 responses [11]. The intramuscular immunization with cDNA has been described to induce clear Th1 responses [12], partially due to CpG motifs within bacteria-derived plasmids [13].

To study the influence of such modifying factors we chose filarial tropomyosin as a model antigen, a protein described by several authors to induce protection against nematode L3. Tropomyosin is a microfilament-protein with a α-helical coiled-coil structure, present in all types of eukaryotic cells [14]. It occurs in several tissue specific isoforms, and is most

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abundant in muscles. In both vertebrates and invertebrates it plays a central role in the regulation of cell motility [15]. In filarial nematodes, tropomyosin is not only present in muscles, but was also found in the cuticle of microfilariae and L3 of *Onchocerca volvulus* [16]. Recently, tropomyosin was described as a potent allergen of invertebrates, e.g. shrimps, mites and insects [17,18] that can provoke food and airway allergies.

Previous studies described the protective capacity of filarial tropomyosin [19–21] and tropomyosin of the intestinal nematode *Trichostrongylus colubriformis* [22], but did not address the effector mechanisms leading to protective immunity. Generally, it is believed that filarial nematodes are attacked by mechanisms of antibody dependant cellular cytotoxicity (ADCC). Elegant studies in mouse model systems have described IgE responses as pivotal for protective anti helminth immune responses induced by irradiated larvae [23]. Thus, it appeared attractive to re-evaluate a surface associated, IgE inducing candidate antigen with respect to potential effector mechanisms in a natural host–parasite-association.

2. Materials and methods

2.1. Cloning and expression of A. viteae tropomyosin

Sequence information from immunoaffinity-purified protein [21] was used to design oligonucleotide primers. A fragment of 612 bp was amplified by PCR from *A. viteae* female cDNA, labeled with digoxigenin (Roche, Mannheim, Germany) and used to screen an *A. viteae* female cDNA library in lambda ZAP. Out of 12 positive clones, one full-length clone of 915 bp was sequenced and subsequently used for further studies. The sequence of the *A. viteae* clone was deposited in GeneBank under the accession number: AF000607. The cDNA sequence was subcloned into the *Bam*HI and *Hin*dIII sites of the expression vector pQE30 yielding polypeptides with a N-terminal six-histidine tag (Qiagen, Hilden, Germany). The recombinant plasmid was transformed into DHFα competent *E. coli* and screened for expression by SDS-PAGE after induction with 1 mM IPTG. The recombinant *E. coli*-expressed protein, termed rAvTropo, was purified under non-denaturing conditions by affinity chromatography using Ni-NTA resin (Quiagen, Hilden, Germany) and dialyzed against PBS/0.1% Triton X-100, pH 7.4 and subsequently against PBS, pH 7.4.

2.2. Preparation of gel-eluted antigen

Worm derived *A. viteae* tropomyosin (wdAvTropo) was prepared by electroelution from SDS-PAGE-separated adult worm extracts. Adult female worm extracts were prepared in PBS, pH 7.4 by homogenization and subsequent sonication for 2 min (Sonifyer Sonoplus HD 200, Bandelin, Berlin, Germany) and centrifugation (10 min, 12,000 × *g*). The position of the tropomyosin band was determined by Western blot analysis of strips of the gel with an anti-*A. viteae* tropomyosin mAb (Sereda et al., unpublished data) and the protein was excised out of the SDS gel after copper staining [24] Subsequently, the protein was electroeluted with a BioTrap BT 1000 device (Schleicher & Schuell, Dassel, Germany). Eluted native tropomyosin was dialyzed twice against PBS, pH 7.4.

2.3. Preparation of DNA vaccine

The cDNA sequence of *A. viteae* tropomyosin was subcloned into the *Eco*RI/*Apa*I restriction sites of the mammalian expression vector pcDNA 3.1+ (Invitrogen, Karlsruhe, Germany). The recombinant plasmid pcDNA/AvTropo was transformed into competent *E. coli* JM109 and plasmid DNA from a 100 ml liquid culture was purified with NucleoBond Plasmid Maxi Kit (BD Biosciences Clontech, Palo Alto, CA, USA) according to the manufacturer's instructions. The recombinant plasmid DNA was resuspended in TE buffer (10 mM Tris pH 7.4, 1 mM EDTA pH 8.0) and stored at −20 °C. Expression of *A. viteae* tropomyosin was analyzed in vitro by transfection of COS7 cells with pcDNA/AvTropo. 2×10^5 COS7 cells/well in a 6-well plate were cultured in 2 ml Dulbeccos MEM medium (Biochrom, Berlin, Germany) supplemented with 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin and L-glutamine (all from Biochrom, Berlin, Germany). Cells grown to 50–70% confluence were transfected by addition of a solution of 1 μg plasmid pcDNA/AvTropo or pcDNA 3.1^+ in 3 μl of FuGENE 6 (Roche, Mannheim, Germany) and 97 μl of serum free MEM medium. After 30 min of incubation the mixture was evenly, drop-wise, added to the cell culture wells and incubated for 48 h after a gentle mix. To determine transfection efficiency, cells were transfected in the same way with a control plasmid pEGFP-N1 (Clontech, Heidelberg, Germany) encoding the EGFP protein and monitored for fluorescence under UV-light. The transcription of AvTropo sequence was analyzed by RT-PCR using specific *A. viteae* tropomyosin primers (Tropo fw 5′ ATG GAT GCG ATC AAG AAA AAG 3′, Tropo rev 5′ ATA TCC AAA AAG TTC TTG GAA GG 3′).

2.4. Western blot to detect of A. viteae tropomyosin expressed in COS7 cells

Tropomyosin expressed in transfected mammalian cells was detected by immunoblot using a monoclonal antibody specifically recognizing *A. viteae* tropomyosin, but not the vertebrate protein (Sereda et al., unpublished data). A 12% SDS-PAGE was run using lysates of 2×10^5 transfected or untransfected, respectively. Subsequently, proteins were blotted onto Protran® nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany) for 45 min in a semi-dry blot chamber (LSM, Dossenheim, Germany). Membranes were then trimmed and blocked with 5% skimmed milk in TBS pH 7.4. After washing three times, 5 min with TBST, membranes were incubated 1 h at RT with undiluted hybridoma culture supernatant. After subsequent washing with TBST an