Vaccine 29 (2011) 3162–3168

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Identification and characterization of protective epitope of *Trichinella spiralis* paramyosin

Junfei Wei, Yuan Gu, Jing Yang, Yaping Yang, Shaohua Wang, Shijuan Cui, Xinping Zhu*

Department of Parasitology, School of Basic Medical Sciences, Capital Medical University, Beijing 100069, PR China

ARTICLE INFO

Article history:
Received 29 December 2010
Received in revised form 10 February 2011
Accepted 15 February 2011
Available online 5 March 2011

Keywords:
Trichinella spiralis
Monoclonal antibody
Phage-displayed peptide library
Mimotope
B-cell epitope

ABSTRACT

Trichinella spiralis paramyosin (*Ts*-Pmy) is a protective antigen that induces partial immunity against *T. spiralis* infection in mice. To identify protective epitope of *Ts*-Pmy, a monoclonal antibody (mAb) 7E2 against the recombinant protein was generated, which partially protected against *T. spiralis* infection following passive transfer. The mAb was used to screen a random phage-displayed peptide library. Ten positive clones were identified, most of which matched amino acids 88–107 or 108–127 of *Ts*-Pmy. Expression of overlapping fragments of *Ts*-Pmy in *E. coli* confirmed that region 88–107 was specifically recognized by 7E2. A peptide based on this epitope region (YX1) was synthesized and shown to compete with native *Ts*-Pmy for binding to 7E2. Mice immunized with KLH-conjugated YX1 were protected against *T. spiralis* larval challenge. The identification of a protective epitope within *Ts*-Pmy highlights the possibility of developing a subunit vaccine against *T. spiralis* infection.

1. Introduction

Trichinellosis is one of the most important foodborne zoonotic diseases worldwide [1]. *Trichinella spiralis* is the major species that infects human as well as more than 100 other mammal species [2]. Human infection occurs by ingesting raw or undercooked meats such as pork, horse meat, and meat from game animals [3,4]. It has been estimated that more than 11 million people could be infected worldwide [5]. Increasingly, trichinellosis is regarded as an emerging or re-emerging disease in some parts of the world due to changes in diet and cooking practices [2,6]. Human outbreaks have been regularly reported in different areas of the world [7–9]. The prevention of *Trichinella* infection is problematic due to the wide distribution of domestic and wild animal reservoirs and the difficulties in diagnosing the disease [10,11]. Therefore, development of vaccines against infection in domestic animals and humans would provide a useful tool in order to control the disease.

Vaccines based on crude larval extracts [12], excretory–secretory (ES) products [13], DNA [14] or recombinant proteins [15,16] have been reported in animal models. Over the last decade, several proteins which are either secreted by *T. spiralis* or involved in establishing parasitism/infection have been developed as recombinant vaccine components, and partial protection against *Trichinella* larvae challenge has been achieved [16,17]. However, because the parasite's antigens change during its development in the host, and because multiple molecules are involved in establishing parasitism, it is difficult to elicit high levels of protection or sterile immunity with a single antigen. Thus, multivalent proteins may be required to generate an effective vaccine. One approach is the design of a subunit vaccine incorporating the epitopes of several protective antigens [18,19]. When combined as a polyprotein only antigenic determinants (epitopes) relevant for protection would be included, thereby avoiding the induction of harmful or cross-reactive immune responses [20,21]. A prototype for such a vaccine is under development and clinical testing for leishmaniasis [22,23].

Invertebrate paramyosin is a major structural component which assembles with myosin and core proteins to form the macromolecular thick filaments [24]. The protein is constitutively expressed in various developmental stages of helminth parasites and is an abundant antigen on the outermost layer of the parasite [25,26]. Additional evidences have shown that, in addition to being a structural protein, paramyosin is also an immunomodulatory protein which plays an important role in host–parasite interactions during helminth infections [27,28]. As an immunogenic target for protective immunity, paramyosin has been identified and characterized in a variety of parasites, including *Schistosoma mansoni* [29], *Schistosoma japonicum* [30,31], *Taenia solium* [32], *Taenia saginata* [33] and *Brugia malayi* [34], and has been proposed as a vaccine candidate against parasitic helminth infection. In our previous work, a full-length cDNA encoding *T. spiralis* paramyosin was cloned by immunoscreening a cDNA library with protective immune serum. BALB/c mice vaccinated with recombinant *Ts*-Pmy protein (r*Ts*-Pmy) develop a Th2-biased immune response and are partially protected against *T. spiralis* larval challenge [16,17].

* Corresponding author. Tel.: +86 10 8391 1436; fax: +86 10 8391 1436.
E-mail address: zhuxping@ccmu.edu.cn (X. Zhu).

0264-410X/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2011.02.072

In the present study, we describe a monoclonal antibody that specifically recognizes r*Ts*-Pmy and protects naive mice against *T. spiralis* infection following passive transfer. By immunoscreening a phage display library and overlapping fragments of r*Ts*-Pmy with the protective mAb 7E2, a protective epitope of *Ts*-Pmy was identified.

2. Materials and methods

2.1. Parasites and antigen preparation

T. spiralis (ISS 533) parasites were originally isolated from a swine source and maintained by serial passage in ICR mice in our laboratory.

Adult worms were collected from intestines of mice 5–7 days after experimental infection. The newborn larvae (NBL) were obtained from fertile female adult worms cultured *in vitro* overnight at 37 °C. Muscle larvae (ML) were recovered from the digestion of muscle from infected mice using a standard pepsin–hydrochloric acid digestion method as described by Dennis et al. [35]. Crude somatic extracts of adult worms, NBL and ML were prepared by conventional methods [36].

2.2. Experimental animals

Female BALB/c mice, aged 6–8 weeks and free of specific pathogens, were provided by the Laboratory Animal Center of the Academy of Military Medical Sciences in China.

2.3. Mouse antisera

Infected mouse sera were obtained from BALB/c mice 45 days post-infection with 500 *T. spiralis* ML.

2.4. Expression of recombinant proteins

The cDNAs encoding full-length *Ts*-Pmy (1–2655 bp) and the N-terminal fragment *Ts*-Pmy-N (1–966 bp) were subcloned into the pET-28a (+) vector (Novagen, USA). The recombinant proteins were expressed in *E. coli* BL21 (DE3) with a his-tag at the C-terminus and purified with Ni-affinity chromatography (Qiagen, USA) according to the manufacturer's instructions.

2.5. Development and characterization of mAb 7E2

Monoclonal antibodies (mAbs) against recombinant *Ts*-Pmy-N (r*Ts*-Pmy-N) were produced using a standard procedure [37,38]. Briefly, splenocytes from a mouse immunized three times with 50 μg of r*Ts*-Pmy-N were fused with SP2/0 cells at a 5:1 ratio with PEG1500 (Sigma–Aldrich Co. Louis, USA). The culture supernatant was screened for antibodies against r*Ts*-Pmy-N and crude somatic extracts of ML by ELISA and Western blot analysis. The positive hybridoma clones were selected and further cloned three times by limiting dilution. A hybridoma cell strain 7E2, with stable growth and secretion of high antibody titers was selected for further analysis. Hybridoma cells were inoculated into the peritoneal cavity of BALB/c mice after pristane treatment (Sigma–Aldrich Co. Louis, USA) to produce ascites. The ascites fluid was collected and mAb was purified by affinity chromatography using a Protein A-sepharose 4FF column (GE Healthcare, USA), according to the manufacturer's instructions.

The antibody titer of culture supernatant and ascites were determined by indirect ELISA. The isotype of mAbs was determined with a Mouse Monoclonal Antibody Isotyping Kit (Gibco-BRL, USA). Western blot analysis was performed to confirm the specific binding of mAb 7E2 to r*Ts*-Pmy (20 ng) or to native *Ts*-Pmy in the crude somatic extracts of ML, NBL or adult worms (2 μg each).

2.6. Passive immunization using mAb 7E2

To determine the protective efficacy of mAb 7E2 against *T. spiralis* infection, 6 female BALB/c mice received 500 μg of 7E2 intravenously in 0.1 ml phosphate-buffered saline (PBS). Additional groups of mice received *T. spiralis*-infected mouse sera (0.1 ml) or PBS as controls. Two hours after injection, mice from all groups were challenged orally with 400 *T. spiralis* ML. On day 4, all mice received a boost injection containing the same amount of mAb, infected sera or PBS. Reduction in worm burden was evaluated by counting the number of ML recovered from mice sacrificed 45 days post-infection.

2.7. Panning a random phage-displayed peptide library

To determine the epitope recognized by mAb 7E2 on *Ts*-Pmy-N, the Ph.D.-12 Phage Display Peptide Library (NEB, Beverly, MA, USA) containing random combinations of 12-mer peptides was screened with 7E2 according to the manufacturer's instructions with minor modifications. Briefly, a 6-well plate (Costar, USA) coated with purified 7E2 (100 μg/ml) was incubated with 1 ml of diluted phage (approximately 1.5×10^{11}). The antibody-bound phages were eluted with 1 ml of elution buffer (0.2 M Glycine–HCl, pH 2.2), titrated, amplified in *E. coli* ER2738 for 4.5 h and harvested by precipitating with PEG/NaCl (20% PEG-8000, 2.5 M NaCl). These primary positive phage clones were biopanned for another two rounds under more stringent conditions (such as reducing the amount of 7E2 used to coat the plates and a shorter phage binding time) to obtain single positive phage clones.

2.8. Analysis of positive phage clones

Positive phage clones were tested for their specific binding to mAb 7E2 by indirect ELISA and Western blot. Briefly, a 96-well plate coated with 7E2 (10 μg/ml, 150 μl/well) was incubated with 10^{10} phage particles of the positive clones, and probed with HRP-conjugated anti-M13 antibody (#27-9411-01, Pharmacia, USA). For Western blot, the phage particles (10^{11}) from positive clones were transferred onto a PVDF membrane, incubated with 7E2 (0.1 μg/ml) at 4 °C overnight, probed with HRP-conjugated goat anti-mouse IgG (Sigma–Aldrich Co. Louis, USA) and developed with diaminobenzidine (DAB) (Amersham, USA).

The single strand phage DNAs of the positive phage clones were extracted from the phage particles and sequenced with the phage flank 96-primer (NEB, Beverly, MA, USA). The phage peptide sequences were deduced from the DNA sequences and aligned with the *Ts*-Pmy sequence using the MEGALIGN programme in DNAStar.

2.9. Peptide synthesis and binding to mAb 7E2

The peptides YX1 and YX2, based on the amino acid regions 88–107 and 108–127 of *Ts*-Pmy (the most possible regions as epitopes recognized by mAb 7E2 based on the positive peptide sequences alignment with backbone *Ts*-Pmy-N), were synthesized by Beijing AVIVA Systems Biology Company. To increase their antigenicity and immunogenicity, the synthesized peptides were conjugated to BSA or KLH. To determine their binding abilities to 7E2, the BSA-conjugated peptides were coated on 96-well plates (10 μg/ml, 100 μl/well), and 7E2 (1 μg/ml, 100 μl/well) was added. To determine the competitive binding of the synthesized peptides with r*Ts*-Pmy-N to 7E2, 7E2 (1 μg/ml) was pre-incubated with various amounts of peptides (100 μg, 10 μg, 1 μg, 0.1 μg, 0 μg) at 37 °C for 2 h before adding it to the r*Ts*-Pmy-N-coated plate. Western