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Short communication

Protection of pigs against *Taenia solium* cysticercosis by immunization with novel recombinant antigens

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ABSTRACT

Recombinant antigens from the oncosphere stage of the parasite *Taenia solium* were expressed in *Escherichia coli*. The TSOL16, TSOL45-1A and TSOL45-1B recombinant antigens, each consisting of fibronectin type III (FnIII) domain S, were produced as fusion proteins with glutathione S-transferase (GST) and maltose binding protein (MBP). Groups of pigs were immunized twice with the GST fusions of the antigens and boosted a third time with the MBP fusions prior to receiving a challenge infection with *T. solium* eggs. The TSOL16 antigen was found to be capable of inducing high levels of immunity in pigs against a challenge infection with *T. solium*. Immunological investigations identified differences in immune responses in the pigs vaccinated with the various antigens. The results demonstrate that the TSOL16 antigen could be a valuable adjunct to current porcine vaccination approaches and may allow the further development of new vaccination strategies against *T. solium* cysticercosis.
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1. Introduction

Cysticercosis in humans occurs following infection with the cestode parasite *Taenia solium* and is a major cause of neurological disease worldwide [1]. It is associated with poor living standards and poor sanitation, occurring in developing countries where free-roaming pigs and the lack of latrines contribute to transmission of the parasite from pigs to humans. Vaccination of pigs has been proposed as a potential tool to control transmission of *T. solium* from pigs to humans, in order to reduce the incidence of human neurocysticercosis [2,3]. A recombinant subunit vaccine, the TSOL18 antigen, has been shown to be highly effective in preventing infection of pigs in controlled experimental trials [4,5]. The TSOL18 vaccine is also highly effective as a porcine vaccine against naturally acquired infection with *T. solium* [6].

Other recombinant antigens have also been cloned from the larval oncosphere stage of the *T. solium* parasite. These include a family of related antigens, designated TSOL45, that have been identified as protein isoforms, some of which result from alternatively spliced mRNA transcripts in the oncosphere [7]. Analyses of the TSOL45 mRNAs have identified a variety of oncosphere proteins encoding two, one or no fibronectin type III (FnIII) domains. One of these gene products, TSOL45-1A, that is not alternatively spliced and contains two FnIII domains, has been shown to protect pigs against experimental infection with *T. solium* [4,5]. Other antigens encoded by the TSOL45 gene family have not yet been evaluated for their ability to protect pigs against infection with the *T. solium* parasite.

The TSOL16 antigen is a third *T. solium* antigen type that has been cloned from oncospheres and the encoding gene has been characterized [8]. It was isolated from *T. solium* following demonstration of the ability of a homologous recombinant antigen, To16, to confer protection of vaccinated sheep against a related parasite, *Taenia ovis* [9]. TSOL16 appears to be specifically expressed in the oncosphere life cycle stage of *T. solium* [10] and is associated with penetration gland cells [11].

Although the development of a porcine vaccine based upon the TSOL18 antigen is at an advanced stage, nevertheless it remains important to evaluate the potential for other antigens to protect pigs against *T. solium*. For example, widespread application of a vaccine based on a single immunogen could potentially select for genetic variants of *T. solium* having reduced susceptibility to the vaccine. Application of a vaccine incorporating multiple, antigenically unrelated immunogens would be expected to reduce the likelihood of selection of resistant parasites, in a manner analogous to the use of different anthelmintics to reduce selection for resistance [12]. Currently available evidence [13] does not suggest

Abbreviations: ELISA, enzyme-linked immunosorbent assay; FnIII, fibronectin type III; GST, glutathione S-transferase; MBP, maltose binding protein.

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that genetic variability in the TSOL18 protein would be a problem during the initial application of the TSOL18 vaccine, however evaluating the ability of other recombinant proteins to complement TSOL18 would add to the potential reliability of vaccination as a control measure for *T. solium*.

The aims of this study were to evaluate whether the TSOL16 protein could be used to protect pigs against infection with *T. solium* and to determine whether a protein related to the TSOL45-1A antigen and encoded by a splice variant lacking one of two FnIII domains (TSOL45-1B) retains the ability to protect pigs against cysticercosis.

2. Materials and methods

2.1. Preparation of recombinant antigens

The TSOL16 cDNA was originally cloned from *T. solium* oncosphere mRNA as described in [8]. Two related TSOL16 cDNAs were first isolated, designated TSOL16A and TSOL16B, which differed at two positions in their predicted amino acid sequences [8]. The TSOL16A cDNA was selected for expression in *Escherichia coli* since the substituted amino acids were identical in sequence to To16 from *T. ovis*, a related antigen that has been previously shown to be host protective in sheep [9]. The encoded TSOL16A protein contains hydrophobic amino acids within a predicted secretory signal at the N-terminus. In order to enable efficient expression of the TSOL16A protein in *E. coli*, PCR amplification was used to produce a cDNA construct encoding a modified form of the antigen that lacked the 16 N-terminal amino acids of the secretory signal. The procedure that was followed is similar to that outlined in [14] and utilized the following PCR primers: 5′CCG GAA TTC GAT GGA TTC GGT GAA TTT GGC G3′; 5′CCG CTC GAG CAT GCA ATG GAA TCC CAG AAG3′. This truncated TSOL16A cDNA (herein referred to as TSOL16 with respect to the cDNA and encoded protein) was cloned directionally into the *Eco*RI and *Xho*I sites of pGEX-1TEX and transformed into *E. coli* JM109 strain by electroporation. Use of the pGEX plasmid allowed expression and purification of TSOL16 as a fusion with glutathione *S*-transferase (GST) [15].

The truncated TSOL16 cDNA was excised from pGEX-1 by digestion with *Eco*RI and *Xho*I, and cloned into *Eco*RI/*Sal*I-digested pMAL-C2. The pMAL-C2 plasmid allowed expression and purification of TSOL16 as a fusion with maltose binding protein (MBP) [16]. The plasmid construct was transformed into *E. coli* JM109.

The TSOL45-1A protein was cloned into the pGEX and pMAL-C2 plasmids, and expressed in *E. coli* as a fusion protein with GST and MBP as described in [4]. The TSOL45-1A fusion proteins lacked 16 N-terminal amino acids that encoded a predicted secretory signal.

The TSOL45-1B cDNA was originally cloned from *T. solium* oncosphere mRNA as described in [7]. TSOL45-1B lacked exon II of the *TSOL45-1* gene. PCR amplification was used to produce a cDNA construct that encoded a protein also lacking the 16 N-terminal amino acids of the secretory signal. The following PCR primers were used to amplify TSOL45-1B for cloning into pGEX and pMAL as described above: 5′CCG GAA TTC GGA AAC CAC AAG GCA ACA TC3′; 5′CCG CTC GAG GGA AAT GGG CAT TGA CCG3′.

E. coli cultures expressing TSOL16, TSOL45-1A and TSOL45-1B were prepared and recombinant fusion proteins were purified as detailed in [14].

Freeze-dried aliquots of antigens were prepared by the addition of Quil A adjuvant (1 mg per dose) and a sixfold (w/w) amount of maltose as a stabilizing agent for transport to Lima, Peru, where the vaccine trial was conducted. Aliquots of GST and MBP, for use as negative controls, were also prepared for the vaccine trial. The antigens were reconstituted in sterile de-ionized water immediately prior to vaccination of pigs.

2.2. Pig vaccination

The purified GST and MBP fusions of TSOL16, TSOL45-1A and TSOL45-1B were tested in a pig vaccine trial against challenge infection with *T. solium*. The study was reviewed and approved by the Animal Ethics Committee of the School of Veterinary Medicine, Universidad de San Marcos, Lima, Peru. Twenty 8-week old piglets were obtained from a cysticercosis free farm located in Huaral, Lima. Animals were divided into four groups of 5 pigs each. All animals were vaccinated against Classical Swine Fever prior to the start of the trial. Each pig received 200 μg of antigen and 1 mg Quil A (Brenntag Biosector, Denmark) per immunization in a 1 ml dose. Immunizations were given intramuscularly in the right hind-quarter via a 0.9 mm × 38 mm needle and 1 ml syringe (Becton Dickinson, U.K.). Piglets received their first immunization with recombinant antigen prepared as a GST fusion. Pigs received a second, identical immunization approximately four weeks after the first immunization. Two weeks after the second immunization, pigs were given a third immunization with recombinant proteins prepared as MBP fusions. Pigs in the control group received GST in the first two immunizations and MBP in the third, all in the presence of 1 mg Quil A.

Blood samples were obtained from the jugular vein of all animals at weekly intervals from the first immunization until thirteen weeks later using 10 ml vacutainers (Becton Dickinson, U.K.) and 18 gauge needles. Serum was separated by centrifugation and stored at −20 °C.

2.3. Parasites and parasite infections

Pigs were challenged with *T. solium* eggs within a single gravid proglottid as described in [5] two weeks after the third immunization and necropsied approximately 3 months after the last immunization. Four different worms were used for supply of the gravid proglottids. The segments from the four worms were randomly distributed to pigs in the various experimental groups.

Carcass muscle was examined for the presence of cysticerci from the challenge infection by slicing at approximately 3 mm intervals. In carcasses which were heavily infected with cysticerci, the total number in muscle were estimated by selecting a muscle sample (of known weight) from the carcass, determining the number of cysticerci in that sample and estimating the total number in the remaining muscle using its weight.

The Mann–Whitney *U* test was used for comparison of the number of *T. solium* cysticerci found in pigs in different groups immunized with the various antigens. A two-tailed *P* value <0.01 was considered to be statistically significant.

2.4. Serological analysis

Specific antibody levels against TSOL16, TSOL45-1A or TSOL45-1B were determined using an enzyme-linked immunosorbent assay (ELISA) as described in [17]. The level of antibody to the specific parasite antigens rather than to the affinity tag (GST) was measured by coating ELISA plates with parasite antigen fused to MBP. Binding of porcine antibody to the MBP fusion proteins of the recombinant antigens was detected using anti porcine IgG-horse radish peroxidase conjugate (Serotec). Antibody titres were calculated from the highest serum dilution at which the optical density at 450 nm equalled 1.0.

Antigenic cross-reactivity was investigated by direct ELISA and inhibition ELISA as detailed by Assana et al. [18]. Briefly, direct ELISA utilized TSOL18-MBP for coating the ELISA wells and application of anti-TSOL16 serum for investigations into antigenic relatedness. The ability of the heterologous recombinant proteins (TSOL18, TSOL45-1A) to inhibit binding of anti-TSOL16 antibodies to homologous antigen (TSOL16) was investigated by antibody inhibition

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