Vaccine development against the *Taenia solium* parasite

The role of recombinant protein expression in *Escherichia coli*

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*Taenia solium* is a zoonotic parasite that causes cysticercosis. The parasite is a major cause of human disease in impoverished communities where it is transmitted to humans from pigs which act as intermediate hosts. Vaccination of pigs to prevent transmission of *T. solium* to humans is an approach that has been investigated to control the disease. A recombinant vaccine antigen, TSOL18, has been remarkably successful at reducing infection of pigs with *T. solium* in several experimental challenge trials. The vaccine has been shown to eliminate transmission of naturally acquired *T. solium* in a field trial conducted in Africa. We recently reported that the vaccine was also effective in a field trial conducted in Peru. The TSOL18 recombinant antigen for each of these trials has been produced by expression in *Escherichia coli*. Here we discuss research that has been undertaken on the TSOL18 antigen and related antigens with a focus on improved methods of preparation of recombinant TSOL18 and optimized expression in *Escherichia coli*.

*Taenia solium* is a zoonotic parasite that infects pigs and humans. It is prevalent in many developing countries of South America, Africa and Asia,1–5 causing a disease known as cysticercosis. Vaccination of pigs has been investigated as a measure to control transmission of *T. solium* to humans.6–8 Recently, we have demonstrated that a recombinant vaccine, produced in *Escherichia coli*, is highly effective at reducing *T. solium* pig infections under field conditions.9 A number of host protective, recombinant antigens have been investigated as potential vaccines for use in pigs, including the S3P vaccine9 and also antigens cloned from *T. solium* oncospheres. Larval oncosphere antigens have provided the highest levels of protection. Three different protective antigens have been cloned from *T. solium* oncospheres and these are designated TSOL18,10 TSOL45-1A11 and TSOL16.12 Each of these antigens has been shown to induce near complete protection in vaccinated pigs challenged with *T. solium* under experimental conditions.12–15 The TSOL18 antigen has shown the greatest potential. TSOL18 has been assessed in five separate pig vaccine trials, under experimental conditions conducted by four independent groups of scientists in four different countries. In the challenge trials, TSOL18 consistently provided vaccinated pigs with nearly complete protection against *T. solium* infection, achieving 100% and 99.5% protection in Mexico,13 99.9% in Peru,15 100% in Cameroon13 and 99.3% in Honduras.16 In addition, the TSOL18 antigen was shown to completely eliminate transmission of *T. solium* to pigs in a vaccine field trial conducted in the Mayo-Danay district of north Cameroon,17 a region previously identified as having a high prevalence of *T. solium*.6,9 A second, more recent vaccine field trial was undertaken in rural villages in the Morropon province of north-east Peru,4 and again vaccination greatly reduced the total number of parasite cysts in immunized pigs (99.7% reduction compared with control pigs).
The TSOL18 antigen used in the field trial described by Jayashi et al. and in the other laboratory and field-based vaccine trials, has been produced by expression in *Escherichia coli*. The antigen was expressed as a fusion protein with glutathione S-transferase (GST) via a pGEX plasmid vector. The GST fusion partner allows purification of TSOL18 from lysed *E. coli* by affinity chromatography on glutathione sepharose. Prior to the antigen being tested in vaccine trials, expression of the TSOL18 antigen in *E. coli* culture required optimization. Initially the cDNA sequence corresponding to the full open reading frame of the associated *tss18* gene was expressed at only very low levels and the TSOL18-GST protein could not be obtained at a high level of purity. Subsequently, the TSOL18 cDNA was truncated, deleting the N-terminal nucleotides encoding a predicted secretory signal of 18 amino acids. These amino acids contained a high proportion of hydrophobic residues. Removal of these amino acids resulted in substantial increases in the expression of soluble TSOL18-GST by *E. coli*.

We have applied this genetic construct design strategy (removal of short stretches of hydrophobic amino acid residues at the N and C termini of parasite proteins) to improve the expression of related cestode antigens in *E. coli*. This strategy was adopted after it was discovered, serendipitously, that a similar approach improved the stability of a related parasite antigen expressed in *E. coli*. The 45W protein from *Taenia solium* was among the first vaccine antigens found to inhibit binding of anti-TSOL18 antibodies to TSOL18 recombinant antigen: black, TSOL18 (amino acids 65–130) lacking secretory signal; white, N-terminal portion of TSOL18 (amino acids 19–89); gray, carboxy terminal portion of TSOL18 (amino acids 65–130). The two genetic truncations of TSOL18 represented by the white and gray arrows (amino and carboxy portions) are likely to have disrupted the protective, conformational epitopes of TSOL18, since either alone or when combined, they were incapable of inhibiting any detectable reactivity of anti-TSOL18 immune serum to TSOL18, indicating that the host protective epitopes of TSOL18 are conformational.

Figure 1. Diagrammatic representation of the predicted fibronectin type III (FnIII) domain within the TSOL18 antigen. (A) Schematic diagram of the secondary structure and position of β strands in the FnIII domain. Large arrows denote the direction (N to C terminus) of β strands within the antiparallel β-sheets (green, pink) of the FrIII domain. Small arrows represent the amino acid positions of each genetic truncation of the TSOL18 recombinant antigen: black, TSOL18 (amino acids 19–89) lacking secretory signal; white, N-terminal portion of TSOL18 (amino acids 19–89); gray, carboxy terminal portion of TSOL18 (amino acids 65–130). The two genetic truncations of TSOL18 represented by the white and gray arrows (amino and carboxy portions) are likely to have disrupted the protective, conformational epitopes of TSOL18, since either alone or when combined, they were incapable of inhibiting any detectable reactivity of anti-TSOL18 immune serum to TSOL18, indicating that the host protective epitopes of TSOL18 are conformational. (B) Tertiary structure of the FrIII domain. Protein structure predictions were performed using Phyre and Swiss-Pdb Viewer. Reproduced (in part) with permission from Kyngdon et al. ©2013 Landes Bioscience. Do not distribute.
The TSOL18 recombinant antigen that is used as a vaccine comprises of a single, predicted fibronectin type III (FnIII) domain (Fig. 1). An FnIII domain has also been described in all other protective oncosphere antigens that have been identified from other cestode parasites.32 The FnIII domain is found in multi-domain proteins from other eukaryotic organisms including cell adhesion molecules, extracellular matrix proteins and cell surface receptors.33 Fibronectins and tenascins contain multiple tandem repeats of the FnIII domain and are associated with cell adhesion, migration and proliferation as well as wound healing, tumorigenesis and embryonic development.34 The three-dimensional structure of the tenth FnIII module of fibronectin has been determined and contains seven β-strands forming a sandwich of two anti-parallel β-sheets.35

It has been noted that single domains of proteins are more likely to be successfully expressed in *E. coli* than large, multi-domain proteins.36 This observation is consistent with genetic construct design strategies applied to TSOL18 and the other cestode antigens, which resulted in successful expression of the antigens in *E. coli*, most likely since they are relatively simple molecules consisting of one or two FnIII domains and a secretory signal, with some also having a C-terminal transmembrane domain. On this basis it has been possible to modify the genetic constructs to delete these regions of the antigens and retain the conformational epitopes within the FnIII domain which confer host protective immunity (Fig. 1).

Experimental investigations have shown that the use of *E. coli* for TSOL18 antigen production may be a feasible approach for future large scale production of the vaccine. However, under standard culture conditions, TSOL18 is often produced as insoluble inclusion bodies in *E. coli*,37 as is also the case for related cestode

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**Figure 2.** Expression of TSOL18-GST in E. coli. (A and B) Comparison of E. coli cultures expressing TSOL18-GST that were incubated at different temperatures and induced using various concentrations of isopropyl β-D-thiogalactopyranoside (IPTG). SDS-PAGE of insoluble proteins (A) and soluble proteins (B) from bacterial lysates. 1–4, samples from cultures incubated at 30°C and induced with 0.2 mM IPTG and incubated at 37°C; 5, sample from culture induced with 0.2 mM IPTG and incubated at 37°C; 5, sample from culture induced with 0.2 mM IPTG and incubated at 37°C. 2,4.5 μl of IPTG; 6, no IPTG. A, protein markers (kDa). Arrows denote position of TSOL18-GST. C, analysis of samples from an optimized fermentor culture containing E. coli expressing TSOL18-GST. A 10L Biostat B Plus fermentor (Sartorius) was used in the fermentation and expression was induced with 0.2 mM IPTG when the OD₆₀₀ reached 20 units (0 h in [C]). The culture was maintained at pH 7.5 and 50% dissolved oxygen (DO) concentration. The OD concentration was maintained using a cascade of agitation between 200–800 rpm and air supplied at 0–20 l/min via a Biostat Bplus micro-DGU. Pure oxygen was blended into the air at high cell densities and was regulated via the control unit. Temperature was set at 37°C and reduced to 30°C at the initiation of induction with IPTG. Super broth (15 g soy peptone, 20 g yeast extract, 5 g NaCl and 100 mg ampicillin per liter) was used in the fermentation. Super broth feed (containing 20% glycerol) was added at 5 ml/min when O₆₀₀ concentration reached 3 units. Half hourly samples were removed from the fermentation and used to determine OD₆₀₀ values of culture samples separated by SDS-PAGE. Following removal of medium by centrifugation, lysis by sonication in phosphate buffered saline and removal of insoluble solids by centrifugation at 10,000 g, TSOL18 expression yields were determined from the soluble protein fractions of culture samples by scanning densitometry (Molecular Dynamics) of culture samples separated by SDS-PAGE. The percentage of TSOL18-GST in total soluble E. coli proteins was determined by scanning densitometry (Molecular Dynamics) of culture samples separated by SDS-PAGE. Following removal of medium by centrifugation, lysis by sonication in phosphate buffered saline and removal of insoluble solids by centrifugation at 10,000 g, TSOL18 expression yields were determined from the soluble protein fractions of culture samples by scanning densitometry and comparison to standards of known mass on SDS-PAGE.
antigens. To investigate this problem, culture conditions for Escherichia coli expressing truncated TSOL18 fused to GST were initially optimized in shake flasks in order to identify the best conditions in which the antigen was produced. Simple adjustment of E. coli culture conditions, by reducing the temperature of induction from 37°C (Fig. 2A and lane 5) to 30°C (Fig. 2A and lane 1–4), resulted in an increase in the proportion of soluble TSOL18 produced. Increased amounts of soluble TSOL18 in extracts of total soluble proteins from lysed E. coli allow increased recoveries of the antigen by affinity chromatography. Optimization of E. coli culture has led to improved expression of TSOL18 and establishment of reproducible culture conditions in a fermentor, providing consistent yields of the antigen (Fig. 2C).

These investigations have demonstrated that high cell densities, together with the use of TSOL18 expression system, can be achieved using the pGEX/E. coli expression system in a fermentor. Consistent yields and improved solubility of TSOL18-GST were observed following optimization of fermentation conditions (Fig. 2C). We also determined that a balance between cell growth and recombinant protein synthesis is necessary to maintain high expression levels in fermentor culture. Various fermentor operating parameters may require fine tuning to maintain this balance, but use of the pGEX vector and E. coli to express TSOL18 in a fermentor provides a robust means of producing the antigen for large scale production.

The operational characteristics for production of the TSOL18 vaccine by bacterial fermentation have been defined.

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They have enabled preparation of the antigen for use in vaccine trials and provide the basis upon which TSOL18 may be produced on a large-scale. The ultimate goal of these studies relates to enabling the widespread use of the TSOL18 antigen as a veterinary vaccine to reduce human infection with cysticercosis. Attention to the development of strategies for large scale production of the antigen may play an important role in achieving these aims.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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