CESTODE VACCINES

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Abstract. Studies over the past 20 years have clearly shown the potential for developing vaccines against larval cestode infections of man and animals. The important larval cestode infections of man (*Echinococcus granulosus* - hydatidosis: *Taenia solium* - cysticercosis) involve domesticated animals as intermediate hosts in their natural life-cycles. These animals develop strong immunity against reinfection, and immunity can be artificially induced by vaccination with oncosphere antigens. A major stumbling block in developing commercial vaccines against cestodes has been the difficulty in obtaining adequate supplies of these antigens. Recent studies with *Taenia ovis*, a larval cestode causing cysticercosis in sheep, have demonstrated the feasibility of developing commercial vaccines against cestodes using recombinant DNA technology. A cDNA library prepared using mRNA obtained from *T. ovis* oncospheres was used to isolate a clone which expressed *T. ovis* polypeptide antigen 45W as a fusion protein with *Schistosoma japonicum* glutathione S-transferase (GST–45W). GST–45W gave up to 94% protection against challenge infection when used to vaccinate sheep with saponin as adjuvant. The vaccine antigen was shown by SDS PAGE to be unstable, a major disadvantage in subsequent attempts to obtain high yields of antigen for commercial production. The fusion protein has now been stabilized by reducing the size of GST–45W cDNA through deleting 19 carboxyl terminal hydropathic acids, and the resultant fusion protein GST–45W (B/X) was highly host-protective. Another experiment showed that the 45W *T. ovis* polypeptide cleaved enzymatically from GST–45W was still host-protective, suggesting that GST had no influence on the immunogenicity of GST–45W fusion protein. Yields of fusion protein from *Escherichia coli* have been substantially increased by developing methods for solubilizing inclusion bodies of GST–45W (B/X) produced in *E. coli* while retaining immunogenicity of the fusion protein. Experiments demonstrating transfer of protective maternal antibody from vaccinated ewes to their lambs, and vaccination of lambs with circulating maternal antibody, both crucial to developing practical vaccination procedures with GST–45W (B/X), are described. The relevance and application of these studies to development of vaccines against other *Taenia* spp. and *E. granulosus* is discussed.

INTRODUCTION

Studies by many scientists during the previous 20 years have clearly shown the feasibility of vaccination to prevent infection of the intermediate hosts of larval cestodes belonging to the Family Taeniidae (Rickard and Williams, 1982). The most important members of this family which infect man are *Taenia solium* (cysticercosis) and *Echinococcus granulosus* (hydatidosis), and the most important natural intermediate host of these parasites are pigs and sheep, respectively. Previous experiments have clearly shown that vaccination with homologous oncosphere antigens confers a high level of host protective immunity in each of these animals (Lightowlers *et al*, in press). A major factor preventing development of practical vaccines against these parasites has been to secure commercial quantities of host protective antigens. Oncospheres can be derived only from adult tapeworms developed in the definitive hosts (*T. solium*, man: *E. granulosus*, dogs), certainly not a commercial proposition!

Taenia ovis is a closely related parasite with the adult tapeworm occurring in dogs, and the larval stage in sheep. It is by far the most extensively studied parasite with respect to developing a vaccine, and serves as a model for the development of vaccines against the other species.

VACCINATION AGAINST *T. OVIS*

Production of a recombinant *T. ovis* vaccine

Johnson *et al* (1989), reported successful vaccination of sheep against *T. ovis* using a defined, recombinant fusion protein antigen designated GST-45W. This report was the culmination of a three-year project between

scientists in the University of Melbourne, New Zealand Ministry of Agriculture and Fisheries, and Coopers Animal Health (NZ) Ltd. Hatched and activated *T. ovis* oncospheres were used as the source of mRNA to construct a cDNA library in the bacteriophage lambda gt11. Clone 45W was selected using rabbit antiserum containing antibodies to putative oncosphere host-protective antigens of Mr 47-52 kDa. These antigens had been shown to be recognized strongly by sheep antibodies in passively protective antisera, or in lambs protected by maternal antibody. 45W cDNA was subsequently subcloned into a vector (pGEX-1) which expressed the *T. ovis* polypeptide as a fusion protein with *Schistosoma japonicum* glutathione S-transferase (GST-45W). This fusion protein gave 94% protection against challenge infection in sheep vaccinated subcutaneously with the antigen in saponin adjuvant.

Stabilization of the fusion protein GST–45W

Subsequent experiments using SDS PAGE showed the fusion protein to be unstable (Rickard, 1989) which was a serious disadvantage in attempting to obtain high yields of antigen for commercial production. O'Hoy *et al* (unpublished results) have now modified 45W cDNA to produce a stable fusion protein, GST–45W (B/X), which is highly immunogenic. Terminal regions of 45W cDNA were removed using various restriction endonucleases in such a way that it allowed expression of most of the full length fusion protein (total 238 amino acids). These modified cDNA sequences were subcloned into the vector pGEX–2T and expressed in *Escherichia coli* strain JM101. Of the four fusion proteins prepared, the one coded by 45W cDNA cut with Bam HI and Xho 11 [GST–45W(B/X)] gave 87% protection in sheep and was shown to be stable in SDS PAGE. Subsequent vaccination experiments with this fusion protein have given consistently between 90 and 100 per cent protection in sheep.

Immunogenicity of *T. ovis* polypeptide 45W B/X

Johnson *et al* (1989) showed that the beta-gal fusion protein produced by lambda gt11 (beta-gal–45W) were not host-protective. It has been suggested that the beta-gal may in some way unfavorably influence the interaction of 45W with the immune system, or that GST contributes positively in some way to its immunogenicity (Rickard, 1989). The plasmid pGEX–2T (Smith and Johnson, 1988) not only allows expression of cDNA clones as fusion with GST, but in addition contains the recognition site for the protease thrombin, thereby allowing for cleavage of the *T. ovis* cDNA encoded polypeptide from the GST carrier. 45W cDNA was subcloned into pGEX–2T, transformed into *E. coli* strain JM101 and the expressed fusion protein cleaved with thrombin (O'Hoy *et al*, unpublished). The 45W polypeptide was purified by removing GST by absorption on glutathione-agarose, and was shown in a vaccination trial in sheep to give 92% protection against a challenge infection with *T. ovis* eggs thus showing that GST per se has no influence on the host-protective activity of GST–45W fusion protein.

Solubilization of GST-45W (B/X) using urea

Only a proportion of the total GST–45W (B/X) fusion protein expressed in *E. coli* is soluble, thereby enabling its purification by glutathione-agarose affinity chromatography. The bulk of the fusion protein is insoluble and present in the bacterium as inclusion bodies. Because of this, the yield of fusion protein using only the soluble portion was far from optimal. Dempster *et al* (unpublished) have developed a process for obtaining partially purified soluble GST–45W (B/X) from the inclusion bodies.

Bacteria were disrupted using a high-pressure homogenizer and the inclusion bodies washed with a borate buffer to remove soluble *E. coli* components. The pellet was solubilized in 9M urea and the soluble protein was diluted to the required concentration for vaccination experiments. Some soluble protein was further purified by dialysis and purification on glutathione-agarose. Sheep vaccination experiments showed that both the purified and unpurified solubilized GST–45W (B/X) were highly host- protective (98% and 97%, respectively).

Protection of lambs against *T. ovis* infection via maternal antibody

Lambs can become infected early in life before it is practically feasible to vaccinate

them. It is crucial, therefore, that pregnant ewes can be vaccinated to protect their newborn lambs via colostral antibody. Passive protection of lambs via colostrum has been previously demonstrated in vaccine trials using native oncosphere antigens. For registration purposes it was necessary to perform experiments to demonstrate similar efficacy of recombinant GST–45W antigen.

Two experiments were carried out (Health *et al*, unpublished). In the first, groups of lambs from ewes either vaccinated twice at 1 to 3 weeks and 5 to 7 weeks before lambing, or nonvaccinated ewes, were infected at 6, 9, 12, and 15 weeks of age. Antibody levels monitored by ELISA using GST–45W as antigen were similar in 3–week-old lambs as in ewes prior to parturition, but fell exponentially until 18 weeks when there was no difference from control lambs. Lambs from vaccinated ewes and infected at 6 weeks of age were 92% protected against infection, maternal antibody protection beyond that time was not useful (57% to 15%).

The second trial was designed to determine whether or not lambs could themselves be vaccinated while they had protective levels of maternal antibody in their serum. Ewes were similarly vaccinated or not as in the first experiment, but in this case half of the lambs from vaccinated ewes were themselves vaccinated at 6 and 12 weeks of age. All lambs were challenged with *T. ovis* eggs at 15 weeks of age. Measurement of antibody levels in lambs showed good responses to the vaccination procedure, and vaccinated lambs were 99% protected against infection compared with control lambs. Numbers of cysts in the nonvaccinated lambs from vaccinated ewes showed again that protection from maternal antibody was of no value by 15 weeks after birth.

RECOMBINANT VACCINES FOR OTHER TAENIID CESTODES

Previous studies have shown clearly that oncosphere antigens from species of cestodes important in man (*T. solium*, *T. saginata*, *E. granulosus*) can be used to effectively vaccinate their intermediate hosts against infection (Rickard and Williams, 1982; Lightowlers *et al*, in press). There is no *a priori* reason why recombinant vaccines cannot be developed for these species. Other studies have demonstrated that a significant level of cross-protection occurs between a number of different species, although it is never as great as homologous protection (Rickard and Williams, 1982; Lightowlers *et al*, in press). If the DNA coding for host-protective antigens in these other species has sufficient homology with *T. ovis*, then techniques such as hybridization and PCR technology utilizing *T. ovis* 45W cDNA provide an obvious and rapid means of obtaining the homologous cDNA from libraries prepared from mRNA extracted from their oncosphere stages.

CONCLUSION

The success with the *T. ovis* recombinant vaccine has shown the potential for developing practical vaccines to assist in control of the important zoonotic species of taeniid cestode. However, a major problem in pursuing this work has been the difficulty in obtaining funds to carry out the necessary research. *E. granulosus* and *T. solium* are major problems in countries where livestock producers are unlikely for economic reasons to use such vaccines. If these vaccines are to be developed, it will necessitate investment by non-commercial research funding bodies or government instrumentalities, and use of the vaccines incorporated as part of official control programs.

ACKNOWLEDGEMENTS

Studies reported in this paper were carried out with financial assistance from the Australian Meat and Livestock Research and Development Corporation, Melbourne and Metropolitan Board of Works, National Health and Medical Research Council and Pitman-Moore (NZ) Ltd [nee Coopers Animal Health (NZ)].

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