Vaccination Against Cestode Parasites

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Abstract—Lightowlers M.W. 1996. Vaccination against cestode parasites. International Journal for Parasitology 26: 819-824. Cestode parasites are important because they cause production losses, particularly in the sheep, beef and pig meat industries, and because some species are zoonotic parasites which cause serious disease in humans. Research on the development of vaccines to prevent infection with cestode parasites has concentrated on the taeniid cestodes. Two strategies can be adopted for vaccine research: vaccines against infection in the definitive hosts and vaccines for use in the intermediate hosts. The number and accessibility of definitive hosts would favour these as potential targets for vaccines over intermediate hosts, however little success has been achieved in demonstrating immune-mediated resistance to infection in definitive hosts. In comparison, immunity in the intermediate hosts is a prominent feature of the host–parasite relationship in taeniid cestodes. This has favoured the development of vaccines against Taenia and Echinococcus species in their intermediate hosts. This paper reviews the progress which has been made in vaccination against cestode parasites and the prospects for practical application of these vaccines. Copyright © 1996 Australian Society for Parasitology. Published by Elsevier Science Ltd.

Key words: Taenia; Echinococcus; cestode; vaccination.

INTRODUCTION

Cestodes are tapeworm parasites which are of economic and medical importance. Those on which vaccine research has concentrated belong to the family Taeniidae. The taeniids have a 2 host, prey–predator life cycle. Infection in the definitive host with the adult tapeworm is of relatively little direct economic or medical importance. Eggs from the tapeworm are released with the faeces and, following ingestion by a suitable intermediate host, the parasite penetrates the tissues and develops into a larval or metacestode stage. Ingestion of infected tissues by the definitive host completes the life-cycle. Infection with the metacestodes of taeniid cestodes causes production losses, particularly in the sheep, beef and pork meat industries as well as causing the serious medical conditions known as cysticercosis and hydatid disease. The economic and medical importance of the taeniids has stimulated efforts to produce vaccines to prevent transmission of these parasites. The principal economically and medically important cestode species are summarised in Table 1 together with information on species infecting laboratory animals which have been used extensively in vaccine research. This paper briefly reviews progress in development of cestode vaccines. A detailed discussion of earlier research is available in previously published reviews (Rickard & Williams, 1982; Lightowlers, Mitchell & Rickard, 1993).

Do we need vaccines?

Control of transmission of most taeniid cestode species is theoretically simple. For example, transmission of hydatid disease through farm dogs could be achieved by a combination of public education not to feed or allow accesses of dogs to offal, and regular treatment of farm dogs with a cestocidal drug such as praziquantel. Similarly, transmission of Taenia saginata or Taenia solium could be achieved by the thorough cooking of beef and pig meat, treatment of tapeworm carriers with praziquantel, improved standards of sewage disposal and personal hygiene.

Some hydatid control campaigns, such as those in Tasmania and New Zealand have achieved the complete cessation of transmission of Echinococcus granulosus to humans using, principally, public education and dog treatment. However, these
Table 1—Taeniid cestode parasites infecting man and/or livestock animals and those used extensively in laboratory-based research

<table>
<thead>
<tr>
<th>Parasite</th>
<th>Associated disease or colloquial name</th>
<th>Intermediate host(s)</th>
<th>Principle definitive host(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echinococcus granulosus</td>
<td>cystic hydatid disease</td>
<td>sheep, goats, cattle, other herbivores, man</td>
<td>dog</td>
</tr>
<tr>
<td>E. multilocularis</td>
<td>alveolar hydatid disease</td>
<td>microtine rodents, man</td>
<td>fox, dog</td>
</tr>
<tr>
<td>Taenia solium</td>
<td>cysticercosis</td>
<td>pig, man</td>
<td>man</td>
</tr>
<tr>
<td>T. ovis</td>
<td>sheep measles</td>
<td>sheep, goats</td>
<td>dog</td>
</tr>
<tr>
<td>T. hydatigena</td>
<td>tennui</td>
<td>sheep</td>
<td>dog</td>
</tr>
<tr>
<td>T. saginata</td>
<td>beef measles</td>
<td>cattle</td>
<td>man</td>
</tr>
<tr>
<td>T. multiceps</td>
<td>gid</td>
<td>sheep (man)</td>
<td>dog</td>
</tr>
<tr>
<td>T. pisiformis</td>
<td></td>
<td>rabbit</td>
<td>dog</td>
</tr>
<tr>
<td>T. taeniaeformis</td>
<td></td>
<td>rodents</td>
<td>cat</td>
</tr>
</tbody>
</table>

have been long and expensive campaigns in highly educated societies in which there exist excellent veterinary and public health infrastructures. Similar campaigns in other areas have been less successful and, in these situations, the availability of a vaccine would assist in achieving a greater level of effectiveness in prevention of the transmission of hydatid disease.

The effectiveness of the public education/dog treatment approach to cestode control varies depending on the population dynamics and basic reproductive rate of the particular parasite. Thus, hydatid disease was controlled very effectively in New Zealand while, at the same time, Taenia ovis was not effectively controlled (Gemmell et al., 1986; Gemmell, Lawson & Roberts, 1987). The situation with T. ovis control in New Zealand illustrates an important constraint on the use of the public education/dog treatment approach. Prior to the introduction of control measures, T. ovis was hyperendemic with lambs becoming infected shortly after birth, probably while they were developing grazing competence. At the same time, lambs received some degree of passive protection against T. ovis with antibody in colostrum. Lambs developed relatively light infection after which they were largely immune from further infection with the parasite. Following the implementation of T. ovis control, the level of environmental contamination was reduced. This led to the sheep flock having reduced T. ovis infection and relatively little immunity to the parasite. When, occasionally, an infected dog escaped the 6-weekly dosing program, the high fecundity of the worm ensured high levels of local contamination. This combined with the low levels of immunity in the sheep flock led to catastrophic levels of infection in some flocks, referred to as cysticercosis storms. Clearly, had the control program included a vaccine for sheep, the occasional breakdown in treatment of T. ovis-infected dogs would not have led to significant infection in sheep thereby hastening the progress of the control campaign and avoiding the economic consequences of cysticercosis storms.

VACCINATION AGAINST INFECTION IN THE DEFINITIVE HOST

The number of potential definitive hosts for taeniid cestodes such as E. granulosus, T. ovis, Taenia multiceps and Taenia hydatigena are fewer than the numbers of potential intermediate hosts so it would be attractive to target the definitive hosts for vaccination. Despite this, substantial progress has not been made in the development of practical vaccines against adult tapeworms. There is little evidence for the development of protective immunity against the adult tapeworm of taeniid cestodes. Typically, hosts can become super-infected or re-infected after anthelmintic removal of an initial infection.

The lack of a protective immune responses against adult taeniid cestodes is not absolute. Gemmell, Lawson & Roberts (1986) undertook extensive investigations of dogs given multiple cycles of infection, treatment and re-infection with E. granulosus. Sixteen dogs were infected on 8 or 9 occasions. Repeated infection had no effect on the proportion of worms which developed to patency. Each dog remained susceptible to infection for a number of infection cycles after which they became less susceptible. The number of infections required to elicit this limited degree of immunity and the expression of the immunity (number of worms establishing, proportion gravid, growth rate) varied greatly between individual dogs. Five of the dogs showed no reduction in infectivity over the duration of the trial. While some dogs did develop a significant level of resistance to infection. The relative ineffectiveness of the immune response against adult E. granulosus tapeworms does
Table 2—Features of host-protective immunity against *T. ovis* infection in sheep induced by vaccination with native oncosphere antigens compared with recombinant oncosphere antigens. Original data concerning vaccination with native antigens are contained in Rickard & White (1976) and Rickard et al. (1977). Data on the use of recombinant antigens are included in Johnson et al. (1989) and Lightowlers et al. (1992) or are unpublished.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Native antigens</th>
<th>Recombinant antigens</th>
</tr>
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<tbody>
<tr>
<td>level of protection</td>
<td>typically greater than 90%</td>
<td>typically greater than 90%</td>
</tr>
<tr>
<td>duration of protection</td>
<td>single vaccination affords protection for a full year</td>
<td>two vaccinations affords protection for at least 6 months.</td>
</tr>
<tr>
<td>colostral transfer</td>
<td>high levels of protection transferred</td>
<td>high levels of protection transferred</td>
</tr>
<tr>
<td>protection of young</td>
<td>lambs successfully vaccinated in the presence of protective maternal antibody</td>
<td>colostral antibody interferes with active immunisation of lambs, however use of a second protective antigen allows active immunisation in the presence of protective antibody</td>
</tr>
<tr>
<td>protection against field-acquired infection</td>
<td>up to 100% protection</td>
<td>more than 85% protection</td>
</tr>
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</table>

not suggest that vaccination is likely to be effective. However, some experiments which have been performed on vaccination against infection with adult cestodes (reviewed in Lightowlers, 1990) have achieved significant levels of immunity (Turner, Berberian & Dennis, 1933; Turner, Berberian & Dennis, 1936; Herd, Chappel & Biddell, 1975) warranting further research.

**VACCINATION AGAINST INFECTION IN THE INTERMEDIATE HOST**

In contrast to the situation with the definitive host, immunity plays a central role in the regulation of transmission of taeniid cestodes through their intermediate hosts. Concomitant immunity is a prominent feature of infection with all taeniid cestodes which have been investigated. Furthermore, in most species immunity is transferred with colostral antibody from the dam. These features have favoured the development of practical vaccines against *Taenia* and *Echinococcus* in their intermediate hosts.

Much of the ground work which established the essential features of host-protective immune responses against metacestode infection was undertaken in the 1930s and 1940s (Miller, 1931; Kan, 1934; Campbell, 1936, 1938). Subsequently, in the 1960s and 1970s research by Gemmell and Rickard (reviewed in Rickard & Williams, 1982) established that high levels of immunity could be achieved against economically and medically important species of cestode in sheep and cattle.

Following the discovery that sheep could be vaccinated using non-living antigens derived from the oncosphere (Rickard & Bell, 1971), extensive investigations proved the potential for practical application of vaccination in the intermediate host. Subsequently, with the application of recombinant DNA techniques to produce commercial quantities of defined antigens, a vaccine was produced against *T. ovis* infection in sheep (Johnson et al., 1989). Table 2 compares the features of the protective immune responses which had been achieved with the native antigens with those induced by the recombinant *T. ovis* antigens 45W, 16.17 and 18K.

Recently, similar methods which were employed for development of a vaccine against *T. ovis* have been used in the development of a recombinant vaccine against *E. granulosus* infection in sheep (Lightowlers, Lawrence, Maas, Gauci & Heath, unpublished observations). As is the case with *T. ovis*, oncosphere antigens of *E. granulosus* are highly protective against challenge infection with *E. granulosus* eggs in sheep (Heath et al., 1981; Osborn & Heath, 1982). A cDNA library was constructed from oncosphere mRNA and screened with antibodies from vaccinated and immune sheep. One particular cloned antigen, designated EG95, induced high levels of protection (96% and 97%) in 2 independent vaccination trials.

The operational characteristics of the EG95 vaccine will need to be defined before the vaccine could be applied rationally in hydatid control campaigns. Matters which require definition include the duration of protection afforded, effectiveness against different strains or geographical isolates of *E. granulosus*, effectiveness in different host species and the protection of young animals by colostral antibody.
COMMERCIALISATION OF CESTODE VACCINES

Despite the *T. ovis* 45W vaccine having been registered for commercial use for several years, the company which has funded much of the vaccine's development, Mallinckrodt, have not proceeded with release of the product. The major difficulty which has been encountered with commercialisation of the *T. ovis* vaccine is likely to be the case also for vaccines against other *Taenia* and *Echinococcus* species. *T. ovis*, *E. granulosus* and most other taeniid species do not cause substantial direct production losses to the livestock industry directly, but indirectly through the transmission to dogs of a human pathogen. Infection with *T. ovis* is not detected until after slaughter. At this time the owner of the carcase is often not the farmer who raised the stock. Losses which are incurred by sheep meat producers due to *T. ovis* would be reflected in the profitability of the industry and hence the price paid to the farmer for the sheep. But this discounting of the value of sheep for meat production is not felt by individual farmers and the value of the discounting due specifically to *T. ovis* is difficult to quantify. Without individual farmers being able to recognise a commercial advantage to them of vaccinating against *T. ovis* they cannot be expected to spend time and money using a *T. ovis* vaccine. Development of the *T. ovis* vaccine in New Zealand through the 1980s occurred in a climate of nationwide, government imposed control measures for *T. ovis*. Governments in the 1990's have moved away from imposing control of "hydatids" in New Zealand, including *T. ovis*. Hence the political climate for commercialisation of a *T. ovis* vaccine is not as attractive as had been the case. Alternative measures are available to provide a commercial return to farmers for vaccination against *T. ovis*, for example by meat producers paying a premium for vaccinated, *T. ovis*-free sheep. To date no marketing strategy has been put in place sufficient to encourage commercial release of the vaccine.

Similar problems could be expected to be encountered with marketing of a vaccine against *T. saginata*, although the public health significance of this parasite may encourage government participation in control. Vaccines against *E. granulosus* may be even more difficult to market in a purely commercial sense. Here, the major significance of the parasite is not to the livestock industry directly, but indirectly through the transmission to dogs of a human pathogen. Practical application of a vaccine is almost certainly going to require a government imposed control program incorporating use of the vaccine. While this is very likely to occur, no clear commercial market can be recognised prior to governments making a commitment to use the vaccine and yet commitments are unlikely to come from governments until the vaccine has been proven to be a practical proposition, i.e. the operational characteristics mentioned above are assessed. Sources of funding other than commercial resources may be required in order to take the vaccine to a stage where a market is created providing the incentive to attract investment for commercial scale up, registration and production of the vaccine.

*T. OVIS 45W AS A MODEL VACCINE*

Development of the 45W based vaccine against *T. ovis* provided, for the first time, a highly effective, defined vaccine against a parasite, available for use in investigations into aspects of anti-parasite vaccination which may be of general relevance to other parasitic diseases.

**Molecular characterisation of antigen expression**

The use of a defined, recombinant antigen has many advantages over use of poorly characterised antigen extracts or whole organisms (Lightowlers, 1994). Disadvantages might also be anticipated due to the relative antigenic simplicity of individual recombinant antigens. If parasite isolates were to exist which varied at critical host-protective epitopes, these parasites may survive vaccine induced immune responses, leading to a breakdown in the effectiveness of the vaccine in a manner analogous to the appearance of anthelmintic resistance. The gene encoding such antigenic variants could be cloned and the variant included in the vaccine. Molecular techniques suitable for the rapid isolation and cloning of specific mRNA's are simple, provided that the desired gene can be targeted specifically. Design of oligonucleotide primers for reverse transcription polymerase chain reaction (RTPCR) from the known sequence of a recombinant vaccine antigen is one strategy which would be suitable. However, this and other approaches rely on specificity for the vaccine gene alone. This approach may fail if other genes are expressed by the parasite which have DNA sequence homology with the vaccine gene but which do not encode host-protective antigens. DNA sequences specific for the vaccine gene may be identified after characterisation of all other genes which show significant sequence homology. Having such gene specific sequences available also allows the vaccine gene in different parasite isolates to be sequenced prior to wide scale use of a vaccine, thereby anticipating the likelihood of selection of antigenic variants. This approach has been taken with *T. ovis* 45W.

*T. ovis* 45W is a member of a family of genes comprising a minimum of 4 members (Waterkeyn et al., 1995). A close homologue of 45W, designated
45S, differs at 11 of 985 nucleotide positions comprising the full length mRNA. Sheep vaccinated with the protein encoded by this variant gene were not protected against T. ovis infection (Johnson et al., 1989). The cDNA encoding the 45S clone was truncated at both 5' and 3' ends compared with 45W such that the 45W protein contains additional protein sequence at both the NH₂ and COOH ends. For this reason it is unclear whether the failure of the 45S antigen to protect sheep is because of antigenic differences between it and the corresponding protein from 45W, or if it is due to the absence of some of the protein coding sequence from the 45S clone. Nevertheless, gene specific oligonucleotide primers were able to be designed which allow 45W or 45S specific amplification of the genes in RTPCR. Application of 45W gene specific oligonucleotides in Southern blots of T. ovis genomic DNA from different parasite isolates has identified variability in the 45W gene, including variation in the number of copies of the gene (Waterkeyn, Cowman Coppel & Lightowlers, unpublished). This variability may have important implications for the effectiveness of the 45W vaccine since parasites expressing different amounts of the 45W antigen may differ in their susceptibility to anti-45W immune responses.

The 45W gene is not restricted in its expression to the oncosphere (Gauci & Lightowlers, 1995). Expression also occurs in developing cysticerci to 8 weeks of age but not in mature, 10-week-old, cysticerci. In the adult tapeworm, 45W expression does not occur in the anterior section of the worm but occurs more posteriorly in association with the embryonation of oncospheres. Expression is enhanced following hatching and activation of the oncosphere, each of which contain an estimated 59 copies of the mRNA. Expression of the gene in the developing cysticercus may account for the production of prominent anti-45W antibodies in sheep infected with T. ovis (Harrison et al., 1993). Invasion and, perhaps, partial development of oncospheres in an immune, vaccinated sheep could be expected to boost anti-45W immunity since the antigen is apparently being actively produced by the parasite beyond the initial oncosphere stage.

Manipulation of immune responses to the 45W vaccine

The 45W vaccine induces antigen specific IgG₁ and IgG₂ antibodies the level of which correlates with the degree of protection afforded to individual animals (Rothel, Lightowlers, Seow, Wood, Heath & Harrison, submitted). The vaccine provides a useful model for studies on manipulation of the immune response to a defined vaccine antigen. Comparison of saponin and oil adjuvants has found that IgG₂ antibody levels are particularly enhanced by the use of oil adjuvant. The immune responses are also altered by incorporation of recombinant ovine cytokines in the vaccine. Inclusion of recombinant ovine IL-1β increases antibody responses by 5-to 10-fold in sheep vaccinated with 45W and ALOH as adjuvant (Rothel, Lightowlers, Corner, Seow & Wood, unpublished). Continuing research is analysing the characteristics of individual 45W-specific lymphocytes in effenter lymph following cannulation of the prefemoral lymph node draining the site of immunisation.

PROSPECTS FOR VACCINES AGAINST OTHER CESTODES

In all taeniid cestode species in which vaccine research has been performed, high levels of protection have been achieved against metacestode infection. This, and the success achieved with recombinant vaccines against T. ovis and E. granulosus, suggests that practical vaccines against other taeniid cestodes would be possible. T. saginata and T. solium are attractive targets for future development of vaccines. Both are human parasites transmitted through livestock. In both species oncosphere antigens have been shown to induce very high levels of protection against challenge infection with eggs (Rickard, Adolph & Arundel, 1977; Rickard & Brumley, 1981; Pathak & Gaur, 1990). T. solium is largely restricted to developing countries and it is the cause of substantial human morbidity and mortality due to neurocysticercosis. A highly effective vaccine against T. solium cysticercosis in pigs would be a valuable tool for preventing the transmission of the tapeworm to humans. Protection of cattle against T. saginata infection would eliminate production losses in the beef meat industry due to cysticercosis.

CONCLUDING REMARKS

The recombinant vaccines against T. ovis and E. granulosus are the most effective of the defined vaccines which have been developed against parasitic organisms. The prominent role played by immunity in the natural regulation of transmission of taeniid cestodes through their intermediate hosts and the effectiveness of crude antigen extracts as vaccines are features which have facilitated this success. Despite the achievements which have been attained in the scientific area, difficulties exist with wide scale practical use of cestode vaccines. Commercial markets are difficult to define and practical use of the vaccines may require them to be incorporated as part of government regulated, parasite control campaigns.
Vaccines against *T. solium* cysticercosis in pigs and hydatid disease in livestock animals are needed in developing countries where the veterinary and other infrastructures are less than ideal and where the funds available for parasite control are particularly limited. In these situations the vaccines need to be inexpensive, simple to administer, stable under relatively harsh environment conditions and to have long lasting efficacy. None of the vaccine delivery systems which have been used to date with *T. ovis* and *E. granulosus* would be likely to be adequate. Future research will examine the prospects for delivering the vaccines using live recombinant bacterial or viral vectors.

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REFERENCES


