WHAT ARE THE PROSPECTS FOR THE DEVELOPMENT OF A VACCINE AGAINST FASCIOLOSIS?

D.J.L. Williams

Veterinary Parasitology, Liverpool of Tropical Medicine/Faculty of Veterinary Science, University of Liverpool, Liverpool, UK.

Introduction

Fasciolosis is a serious disease of sheep and cattle and occurs throughout the world. The exact economic significance of fasciolosis is difficult to quantify, but the disease is regarded as a major constraint on livestock production and the cost to the global agricultural industry is estimated to be US$2,000 million annually. The causative agents of fasciolosis are *Fasciola hepatica* and *F. gigantica* both of which are transmitted by an intermediate snail host of the species *Lymnea*. Currently fasciolosis can be controlled by the use of chemotherapy and chemoprophylaxis or by destroying snails and their habitat, thereby preventing transmission of the parasite. Flukicidal drugs can be used to treat clinically-affected livestock or used strategically either to prevent disease or to prevent contamination of pasture with fluke eggs. But drug-treatment is expensive and is not always used in the most effective way to prevent disease. To be able to vaccinate sheep and cattle against fasciolosis would be a major advance in the control of the disease, particularly in developing countries where foreign exchange for the purchase of flukicidal drugs is limited.

Research over many years has demonstrated that cattle acquire a degree of resistance to fasciolosis following experimental challenge and following immunisation with attenuated flukes whereas sheep remain susceptible to infection even after repeated exposure or immunisation (Haroun and Hillyer, 1986). However the ability of cattle to acquire resistance after natural exposure to the parasite is less well documented. These findings suggest that a vaccine will have to be based on antigens selected empirically rather than identified by characterising an existing powerful protective immune response. Several groups of enzymes have emerged as potential vaccine candidates as a result of research on the closely related parasites, *Schistosoma* spp. In this short paper two groups of enzymes will be considered, the glutathione -S-transferases and the Cathepsin L proteases. Progress relating to these two enzymes has recently been reviewed by Spithill and Dalton (1998).

Glutathione-S-transferase

The GSTs are a family of iso-enzymes which occur throughout the animal kingdom and are found in a wide variety of different parasites. They are detoxification enzymes, able to convert potential toxic endogenous compounds into less reactive forms. Schistosome GSTs have been shown to protect mice against challenge with *S. mansoni* and *S. japonicum* and therefore in view of the similarity between the schistosomes and *Fasciola* GSTs have been tested for their ability to confer protection against experimental infection. GSTs from adult *F. hepatica* have been isolated and cloned but to date, only the purified native molecules have been used in vaccine trials. GSTs have been shown to confer partial protection in

both sheep and cattle (Table 1). The level of protection induced was affected by the choice of adjuvant used. Most vaccine trials have used Freund's Adjuvant, however this is not licensed for commercial use. In GST vaccine trials in cattle the highest protection was observed using the adjuvant Quil A / Squaline Montanide 80®. Protection was evident at both the level of a reduction in the number of adult flukes recovered from the livers of the vaccinated animals and also in a reduction in the fecundity of the flukes.

Table 1
F. Hepatica molecules used in vaccine trials

Vaccine Molecule	Species Vaccinated	Adjuvant	% Protection	Reference
GST	Sheep	Freund's	57	Sexton et al., 1990
GST	Cattle	Q / A SM80®	69	Morrison et al., 1996
Cysteine Protease	Sheep	Freund's	NS*	Wijjfels et al., 1994
CL1	Cattle	Freund's	42	Dalton et al., 1996
CL1 + Hb**	Cattle	Freund's	52	"
CL2 + Hb**	Cattle	Freund's	72	"
Hb**	Cattle	Freund's	44	"

* NS = No significant reduction in worm burden, but faecal egg output was reduced by 69%
** Hb = *F. hepatica* haemoglobin-like molecule

Cathepsin L proteases

A second group of enzymes that has received attention as potential vaccine candidates is the cysteine proteases and in particular, a sub-group of the cysteine proteases, the Cathepsin Ls. These enzymes are excreted / secreted by *F. hepatica* and have been implicated in facilitating the movement of juvenile flukes through the liver, in acquisition of nutrients derived from host blood and tissues and in immune evasion by cleaving host immunoglobulin thus preventing effector cell attachment. Two Cathepsin Ls - Cathepsin L1 (CL1) and Cathepsin L2 (CL2) - have been characterised and cloned and their vaccine potential analysed.

In a trial in which sheep were immunised with CL1 and CL2 together in Freund's Adjuvant no significant reduction was seen in the fluke worm burden, however there was a significant reduction in the fecundity of the worms (Table 1). In another trial, in which cattle were immunised either with the CL1 and CL2 alone or in combination with another *F. hepatica*-derived protein - a fluke haemoglobin - protection was elicited both at the level of a reduction in the worm burden and in a reduced egg output in the vaccinated cattle (Table 1).

Antibody responses to the CL1 and CL2 in naturally infected cattle.

Recent studies have shown that a major antigen in cattle naturally exposed to *F. hepatica* under conditions of high challenge, is a 28 kda protein. All experimentally infected and 83% of naturally exposed, adult cattle were found to have serum antibodies to this 28 kda protein. This protein has been character-

ised and shown to contain the fluke CL1 and CL2 suggesting that these enzymes are major antigens in naturally exposed animals (Ortiz and Williams, manuscript in preparation). Studies are underway to determine if sheep, naturally exposed to the parasite also mount antibody responses to these enzymes.

Conclusions

The feasibility of developing an anti-fluke vaccine appears to be good in view of the reasonably high levels of protection obtained in trials using defined antigens. Several problems remain however. So far the highest levels of protection have been obtained in cattle, few trials have been carried out in sheep and those that have been reported have not shown the same degree of protection compared to cattle. In view of the greater susceptibility of sheep to fasciolosis, and their apparent inability to develop resistance to the parasite, it is possible that a vaccine which is effective in cattle may be less effective in sheep. Secondly, effective adjuvants which can be used alternatives to Freund's Adjuvant but induce equivalent levels of protection have yet to be identified. Finally, all the trials reported so far have used native protein. Clearly this is not viable for a commercial vaccine. Although all the antigens tested so far for their protective potential are available as recombinant molecules, none have so far been shown to confer protection as effectively as the native proteins. However with new expression systems becoming available it is likely that this will change.

There is a feeling of optimism about the development of a vaccine against fasciolosis. If this is realised, then farmers in the developed and the developing world will have a new and effective weapon in their armoury in the fight against the liver fluke.

References

Haroun, E.T.M. and Hillyer, G.V. (1986). Resistance to fasciolosis - A review. *Veterinary Parasitology,* 20, 63-93.

Spithill, T.W. and Dalton, J.P. (1998). Progress in development of liver fluke vaccines. *Parasitology Today*, 14, 224-228.