Prospects for Vaccines Against Gastrointestinal Helminth Parasites of Ruminants

W.D. Smith
Moredun Research Institute
408 Gilmerton Road
EH17 7JH Edinburgh - Scotland

Introduction

With the notable exception of the one for the bovine lungworm, *Dictyocaulus viviparus*, there are no commercially available vaccines for the control of helminth infections in ruminants. Liver flukes and gastrointestinal nematodes are nearly always controlled by a combination of anthelmintic drugs and pasture management. Although this practice can be extremely effective, it is not possible on certain types of farms and on others it can prevent the best use of the grazing that is available. The situation is threatened by the advent of anthelmintic resistant strains of worms. This is particularly acute and widespread in nematodes of sheep and goats in the Southern hemisphere where, for example, strains of *Haemonchus contortus* exist which are resistant to all three chemical groups of anthelmintic.[6] Fortunately for cattle farmers, the situation is not so serious, although anthelmintic resistant *Trichostronylus axei* and *Fasciola hepatica* have been described and in New Zealand a strain of *Cooperia* was recently isolated which was not susceptible to either oxfendazole or ivermectin.[22] Furthermore, concerns have been raised about anthelmintic residues both in food and in the environment, where adverse effects on harmless or beneficial fauna have been described.

Unfortunately there is no news of completely novel anthelmintic drugs under development; the latest products are variants of known chemical groups. Because of this, the expense involved in developing new compounds and some promising results with immunization against other parasites (e.g. the Australian cattle tick *Boophilus microplus*), several commercial companies are investigating the potential of vaccines against ruminant helminths usually in collaborative projects with government funded scientists. If successful, control by vaccination could offer an escape from most of the difficulties outlined above for anthelmintics. Using specific examples, the present paper will concentrate on some of the strategies that are been used to achieve this goal rather than attempt to provide an up to date list of the various protective antigens that have been discovered for the different species of ruminant helminth.

Vaccine Strategies

Early attempts to immunize ruminants against gut helminths using crude worm homogenates as antigen met with little or no success. It was a similar story with attempts to immunize by ectopic infection, where live worms were administered by an unnatural route as "vaccine" (e.g *Ostertagia* given intraperitoneally) followed by an oral challenge infection to assess any protection conferred.

The first breakthrough came in the 1960s when it was discovered that infection with *Dictyocaulus* larvae which had been attenuated by irradiation could stimulate a high degree of protection against challenge with normal infective larvae. This discovery led to the development of "Dictol", the vaccine which is still sold today. Naturally attempts were immediately made to extend the principle to the gut helminths. Unfortunately it was found that while the method worked well under experimental conditions for the like of *H. contortus* and *T. colubriformis* in sexually mature sheep, in young lambs the protective effect was either too weak or too variable to be a practical proposition. Of course the young, highly susceptible ruminant is the type of animal in which vaccines must work if they are to be commercially viable. For reasons that are still unclear, immunity to *Dictyocaulus* spp can develop rapidly and effectively in the calf or lamb whereas immunity to the gastrointestinal nematodes is acquired much more slowly.

Much research has been and is still being conducted into the mechanisms of naturally acquired immunity to gut helminth infections of sheep and cattle, but it is still not clear what the final effector mechanisms are. Unfortunately the situation seems to be complex involving a combination of local hypersensitiv-

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ity, cell mediated, antibody and inflammatory responses. Obviously if and when these and the respective antigens which trigger them are finally unraveled, attempts can be made to stimulate them by immunization. Any technique which stimulates the natural mechanism will have to overcome the natural unresponsiveness which exists in the young lamb or calf and in the dam around parturition.

The vaccine strategy which is enjoying most success currently ignores the mechanisms of natural immunity but attempts to direct high titre antibody responses toward potentially susceptible targets on the parasite. In the case of blood feeding species, the luminal surface of the intestine has been a particularly rich source of suitable target molecules as exemplified below. Because molecules on the luminal surface of the parasite intestinal cells are not normally recognized by the host during infection, these antigens are classified as "hidden", a feature which raises some unconventional concepts relating to vaccination strategy. However, in the case of *F. hepatica* some success has been achieved using a "homologous" antigen, that is an antigen first shown to be protective in another helminth species. Finally, work with *Taenia ovis* has revealed an example of a protective "natural" antigen. Unlike *Fasciola* and the gastro-intestinal nematodes, it is well known that circulating antibodies play a role in naturally acquired immunity to *T. ovis*, a situation which allowed the identification of the first recombinant protective antigen for any helminth of ruminants.

Examples of "hidden", "homologous" and "natural" protective antigens for ruminant helminths are described in more detail below. The general approach for "hidden" and "natural" antigens has been first to screen candidate protective fractions enriched for the parasite target in preliminary protection trials, second to purify the protective components as much as possible and finally to isolate and express the genes which encode these so that a functional recombinant protein can be produced. As the adult stages of the economically important species of ruminant helminth cannot be satisfactorily cultured *in vitro*, it can be relatively difficult and/or expensive to obtain sufficient quantity of worms for the first two of these steps and large numbers of parasite donor animals are required. This partially explains why so much more work has been done with ovine rather than bovine parasites, the exception being adult *Fasciola* which can be obtained relatively cheaply from the abbatoir. It is generally assumed that if protective antigens can be identified in a nematode species which infects sheep, that it should be relatively straightforward to identify the homologous proteins in the equivalent bovine parasite. Of course the final step of producing a functional recombinant antigen is crucial to the whole approach because a helminth vaccine which consisted of native worm antigen could never be a commercially viable proposition.

Hidden Antigens

Gut membrane proteins

The gut membrane approach was first applied successfully to ticks in research which culminated about two years ago in the launch of the recombinant vaccine against *Boophilus microplus*, the Australian cattle tick.[24] The principle is straightforward. The host is immunized with appropriate gut membrane proteins from a blood feeding parasite and a high titre circulating antibody response is raised. When the parasite subsequently feeds on the host, it ingests antibodies which bind to functional proteins on the brush border of its intestinal cells so that its digestive processes are compromised, leading to starvation, loss of fecundity and weakness so that eventually the parasite disattaches and, in the case of the gastrointestinal species, is swept out of the gut by peristalsis. This technique is showing great promise as a method for controlling the blood feeder, *Haemonchus contortus*, and several different gut membrane proteins or protein complexes have been isolated which give more than 80% reduction in eggs together with greater than 50% protection against worm numbers when tested under experimental conditions.[9, 15, 18] So far all of these seem to be or are associated with various types of protease enzyme and the thrust of the current activity is trying to produce these antigens in recombinant form.

A major advantage of the hidden antigen approach is that because the mechanism of immunity is quite different, it works in situations where natural immunity to *Haemonchus* is weak or ineffective. Thus it has been shown that young lambs,[16, 21] goat kids[7] and periparturient ewes[1] can be successfully immunized and that some protective immunity is even transferred by maternal antibody.[1] On the other hand the fact that the antigens are hidden means that unlike conventional vaccines, immunity is not boosted by infection. At first sight this seems a serious disadvantage because it might seem that frequent immunizations would be required for an effective level of protection to be maintained. Experimental evidence suggests otherwise. The mechanism of protection in lambs immunized by this method mainly affects immature and adult worms whereas incoming larval stages which are not yet blood feeders are largely unaffected.[17] Thus when immunized lambs are subjected to repeated daily infections of larvae, to mimic the situation in the

field, faecal egg counts and adult worm numbers are controlled by the vaccine but the continued presence and activity of the early larval stages stimulates a natural immunity which is capable of replacing the effects of vaccine immunity when this wanes.[17] The hope therefore is that *Haemonchus* can be controlled by a combination of immunizing lambs before weaning and ewes during pregnancy to prevent the periparturient rise in egg count.

Another potential advantage of hidden over natural antigens is that because the host does not respond to them during natural infection, the parasite has not needed to adapt to counteract the host response. Thus they are likely to be conserved, both within and probably between species. Certainly the protective gut membrane aminopeptidase from *Haemonchus*, often referred to as H11, has been shown to be effective against geographically distant isolates of worms and, on an equally practical note, it is effective against anthelmintic resistant strains.[12,17]

It is not entirely clear whether the gut antigen principle can be successfully employed against species of nematode which are not direct blood feeders. The precise diet of economically important species like *Ostertagia* and *Dictyocaulus* is not know, but there are data which show they contain host immunoglobulin, ingested it is presumed with mucus, tissue fluids and/or serous exudates.[11] It remains to be determined whether the amounts consumed are adequate for this vaccination strategy to be effective, but there is at least one encouraging result with *Ostertagia circumcincta*.[19] Incidentally, although Dictol is effective, because it is live and attenuated, it is cumbersome to manufacture and distribute, so that a vaccine for *D. viviparus* based on a defined antigen would be an attractive alternative.

Non gut proteins

Glutathione-S-transferase from *F. hepatica* (described below) is a hidden antigen because it is not recognized serologically by sheep or cattle which have been infected by this parasite.[14]

Homologous antigens

The glutathione-S-transferases (GST) of *F. hepatica* were chosen as candidate vaccine antigens because homologous proteins from *Schistosoma mansoni* and *Schistosoma japonicum* had been shown to be protective in laboratory animal model infections.[10] GSTs are involved in the metabolism of xenobiotics, transport of anionic compounds and the detoxification of lipid peroxides. Sheep or cattle immunized with native GSTs isolated from *F. hepatica* have been protected on average by 49 and 29%, respectively, although the results from individual trials have been quite variable.

It is not clear by what mechanism protection is induced. The simplest possibility is that anti-GST antibody neutralises these enzymes which normally counteract the effects of reactive oxides produced by the inflammatory response of the host.

Even though it has been found that GSTs are unlikely to be useful antigens for *Haemonchus*, there is no doubt that the approach of using DNA or antibody probes to identify protective antigen homologues between parasites will become more and more commonplace and should provide a valuable short cut to the laborious protein fractionation procedures which have mainly been employed to date.

Natural antigens

Technically, the most advanced defined antigen vaccine for ruminant helminths, indeed for any helminth parasite, is that for *Taenia ovis*, where 3 distinct highly protective recombinant antigens have been synthesized. All three are oncosphere antigens which are recognized serologically by naturally infected sheep. The first one to be discovered, designated To45W[8] has been extensively tested in field trials in New Zealand with great success. Two further antigens known as To18 and To16.17 appear to be just as promising.[5] However, *T. ovis* is not a human or sheep pathogen. Although harmless, the cystic stage in meat has been used as a trade barrier for the importation of New Zealand lamb, as it presents an aesthetic problem to the diner. Therefore commercialization of the vaccine has not occurred for political and marketing reasons.[13]

Cathepsin L from *F. hepatica* is an example of an enzyme which is recognized by the host immune response following infection and which can induce high levels of protection when used as a vaccine antigen. Vaccination of sheep with native enzyme reduced *Fasciola* egg production by 70%,[23] whereas one report in cattle claimed 100% protection against eggs.[4]

Interest in this protease was originally aroused when secretions from *Fasciola* were found to degrade immunoglobulin and it was suggested that if the enzyme responsible could be neutralized by antibody, the flukes would become more susceptible to the host response.[3] However flukes recovered from sheep vaccinated with cathepsin L were morphologically normal, suggesting that their development had not been retarded as might be expected if the hypothesis were true. It has been suggested that the parasite uses a cathepsin protease for egg production and that antibody raised by the secreted protease in the vaccine cross reacts with this, interfering with egg synthesis.[20] Support for this hypothesis has come from studies which dem-

onstrate the presence of a cathepsin in the Mehlis gland, a group of cells involved in the formation of the egg shell. If this second hypothesis is correct, luck will have played a large part in the discovery of this antigen!

How Good Do Worm Vaccines Have To Be?

Conventional wisdom suggests that a worm vaccine would have to approach the efficacy of either anthelmintics or bacterial or viral vaccines to be effective. In the case of the gastro-intestinal nematodes of ruminant it is more appropriate to consider a vaccine as an epidemiological tool to maintain low level pasture contamination, rather than a weapon to abolish infection completely. Barnes, Dobson and Barger[2] used a respected mathematical model they developed for simulating *Trichostrongylus* populations in grazing sheep to compare theoretical vaccines of nominal efficacy with conventional control methods based on anthelmintic treatment. They concluded that if the vaccine consisted of a natural antigen, only 60% efficacy in 80% of the flock would bring substantial benefits; if the vaccine was based on a novel antigen, then if 80% of the flock was protected or if it achieved 80% efficacy, it would give better control than a conventional control program.

Conclusions

The prospects for defined antigen worm vaccines are brighter than ever. Scientifically, the concept has been proven for *T. ovis*. In the case of *Haemonchus* and *Fasciola*, it is probably only a matter of time before effective recombinant versions of the known protective native antigens are produced. Hopefully the principles can be extended to other important species. Let's hope it will not be too long before vaccines can be added to the existing methods for controlling helminth infections of grazing ruminants.

Summary

Vaccines for several of the more important species of ruminant helminth are being developed, spurred on by the increasing frequency of anthelmintic resistant strains of worms. Several highly protective antigens have been identified, but so far highly protective recombinant versions of these have only been produced for *Taenia ovis*. Of the gut helminths, *Haemonchus* and *Fasciola* are likely to be the first candidates for defined antigen vaccines. The approaches which have been used to identify the various antigens and likely strategies for their use are discussed.

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