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Developments and hurdles in generating vaccines for controlling helminth parasites of grazing ruminants

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Abstract

As a direct consequence of rising drug resistance among common nematodes of grazing animals, efforts toward state-of-the-art vaccine development have clearly intensified in recent years, fuelled primarily by the advent of newer technologies in gene discovery, by advancements in antigen identification, characterisation and production. In this regard, it is appropriate to review progress that has been made in generating helminth vaccines and in particular, vaccines against common nematodes of production animals for consumption. In like manner, it is prudent to evaluate barriers that have hindered progress in the past and continue to present obstacles that must be solved when utilizing and depending on host immunity to attenuate parasitic infections.

Keywords: Helminths; Vaccines; Hidden antigens; ES antigens; Formulation; Delivery

1. Introduction

Twenty-one years ago, the *Plasmodium falciparum* circumsporozoite gene, which encodes a key target antigen for vaccine development, was cloned and expressed (Dame et al., 1984; Enea et al., 1984). At that time, it was considered a breakthrough in the development of a cost-effective and stable alternative for vaccination against a parasitic disease that affects millions of people in endemic regions throughout the world. Unfortunately, the logical outcome of this work, a viable recombinant vaccine, still eludes researchers today (Graves and Gelband, 2003). This battle to produce efficacious parasite vaccines is not unique to *Plasmodium*. Of the few parasite vaccines that are commercially available, nearly all are based on attenuated organisms. In general, protection wanes upon removal of the immunological agent and as such the host requires repeated natural or artificial boosting to maintain immunity. Even though they do not stimulate sterile immunity as such, these and other potential parasite vaccines could still be useful management tools for controlling helminth disease. With the notable exception of one available parasite vaccine for the bovine lungworm, *Dictyocaulus viviparus*, there are no commercially available vaccines for the control of

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helminth infections in ruminants. Liver flukes and gastrointestinal nematodes are nearly always controlled by a combination of anthelmintic drugs and pasture management. Although these practices can be extremely effective, the advent of anthelmintic resistance indicates that they are not sustainable.

In the last 15 years, considerable progress has been made in identifying candidate vaccine antigens for several important helminth species and many reviews have been published recently (Knox and Smith, 2001; Knox et al., 2003; Lightowlers et al., 2003; Newton and Meeusen, 2003). The present review concentrates on some of the obstacles that must be overcome to contend with the enigmatic problem of obtaining helminth vaccines for ruminants as well as numerous strategies, most notably, targeted antigen discovery, that are being used to circumvent them to achieve the goal. In this regard, we describe some of the protective antigens that have been identified in recent years with emphasis on the most promising discoveries. More complete lists of less recently discovered antigens can be found elsewhere (Emery, 1996), though research on many of these antigens seems to have been abandoned.

2. Vaccine strategies

In the 1960s, 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'', a vaccine, which is still sold today. Attempts were immediately made to extend the principle to the gastrointestinal helminths and, the method worked well in inducing protection under experimental conditions for *Haemonchus contortus* and *Trichostrongylus colubriformis* in sexually mature sheep. However, in field infections the protective effect was either too weak or too variable to be a practical proposition, particularly in young lambs. Attenuated larval vaccines utilise the complex effector mechanisms that constitute naturally acquired immunity, which, for many endoparasites are known to be acquired more slowly in young animals and be subject to disruption during pregnancy, lactation and by poor nutrition.

The vaccine strategy currently enjoying most success largely ignores the mechanisms of natural immunity, but attempts to direct responses towards potentially susceptible targets on or secreted by the parasite. For example, with the cestodes, *Taenia* and *Echinococcus*, molecules on the surface of the delicate oncosphere stage are appropriate targets, whereas for *Fasciola hepatica*, *H. contortus* and *Ostertagia ostertagi* certain proteins excreted or secreted by the parasite are also candidate protective antigens. The latter are known as ES (excretory/secretory) antigens and, because they are recognised by the host immune system following infection, are classified as ''natural'' antigens. However, in the case of blood feeding *Haemonchus*, the luminal surface of the intestine has also been a particularly rich source of suitably effective target molecules.

Examples of ES and gut protective antigens for ruminant helminths are described in more detail below. The general approach for identifying either type of antigen has been first to screen candidate protective fractions enriched for the parasite target in preliminary protection trials, second to purify the protective components as far as possible and finally to isolate and express the genes which encode these so that an efficacious recombinant protein can be produced as a cost-effective vaccine antigen.

As the parasitic stages of the economically important species of ruminant helminths cannot be satisfactorily cultured in vitro, it can be relatively difficult to obtain sufficient quantity of worms for the first of these steps, and to collect enough material to purify the antigen of interest by fractionation of the starting material in step two is even more problematic. Large numbers of parasite donor animals are required, usually making the process very expensive, especially if low yielding ES is the starting material. A recent, sensitive analysis of parasite derived proteins revealed just how many proteins are present in *Haemonchus* ES (Yatsuda et al., 2003). With current immunisation regimes, only a few micrograms of contaminating protein are sufficient to stimulate a strong antibody response, emphasising the difficulties of ascribing protection to any particular molecule. Most of the ES derived protective antigens were obtained by a single simple fractionation step, a process unlikely to purify any molecule from the complex starting material completely. This limitation

should be borne in mind with the protective antigens described below.

The advent of bioinformatics and ''gene mining'' offers the possibility of identifying and synthesising potentially protective parasite antigens directly, without having to isolate their equivalent native proteins first. This approach is more fashionable than the traditional ''fractionate and vaccinate'' process described above. Despite considerable investment, e.g. in vaccines for fleas, certain nematodes and the salmon louse, it has not been successful to date. Part of the problem is that if, as is the norm, the recombinant antigen fails to protect in an animal trial, it is not known whether the original target was an invalid choice or whether in fact a potentially protective antigen had been identified, but was not in the correct format. Even if a partially protective recombinant antigen was identified, it would be impossible to determine its full potential without access to it in native form. Given the expense of vaccine trials in large animals and with so many potential molecules and variants of such molecules to evaluate, it is hard to maintain funder's enthusiasm for any particular antigen unless its native equivalent has been shown to have good potential first. This probably explains why the only successful parasite recombinant vaccine developments to date have derived from the ''fractionate and vaccinate'' approach (Lightowlers et al., 2003).

3. Cestode oncosphere antigens

Technically, the most advanced defined antigen vaccines for any helminth parasite, are those for the cyst stages of various cestode species where the ruminant or pig acts as the intermediate host. Extensive early ground work with these infections showed that naturally acquired immunity was solid and could be reproduced with preparations from the parasite oncospheres (eggs). Following extensive fractionation experiments with native oncosphere proteins to identify the active components, highly protective recombinant antigens were synthesised for *Taenia ovis* and *E. granulosus*. The identification of these antigens subsequently allowed the rapid development of homologous vaccines for the intermediate hosts of *T. saginata*, *E. multilocularis* and *T. solium* (Lightowlers et al., 2003). However, as the importance of all these parasites is mainly zoonotic with disease mostly being restricted to the poor in developing countries, it remains to be seen whether these scientific successes can be translated into either commercial or publicly funded vaccines, especially as control can also be achieved by measures, such as better meat inspection and improved sanitation.

4. Nematode and fluke ES antigens

While it is convenient to compartmentalise helminth protective antigens as originating from the ES or the gut, it should be borne in mind that the precise boundaries between these sources are blurred. ES are obtained by incubating worms harvested from donor animals in simple media, usually for up to 48 h. During this time some parasites may die, releasing gut membrane proteins into the medium in a non-physiological manner. For example, microsomal aminopeptidases have been detected in *Haemonchus* ES (Yatsuda et al., 2003), even though they are known to be integral gut microvillar membrane proteins (Smith et al., 1997). Equally, gut proteins, known to be integral to the microvillar membrane because they can only be extracted with detergent (Smith et al., 1999), could subsequently be cleaved or secreted in a physiological manner so that they or their fragments appear in the ES (Yatsuda et al., 2003).

Cathepsin L from *F. hepatica* is an example of an enzyme which is recognised by the ruminant 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% and egg viability by more than 80% (Wijffels et al., 1994). Whereas one report in cattle claimed 50% reduction in fluke numbers, this effect was improved to 70% in cattle immunised with cathepsin L combined with fluke haemoglobin and it was found that almost 100% of the eggs shed by the vaccinated animals failed to embryonate, suggesting that parasite transmission would be enormously reduced (Dalton et al., 1997).

Striking protective effects have been reported against *Haemonchus*, using preparations enriched for two proteins of 15 and 24 kDa from adult worm excretory–secretory products (Schallig et al., 1997).

These antigens are recognised serologically by infected sheep, and though related to similar molecules in *Ancylostoma* (termed ASPs) their biological function has not yet been determined. At first it was not possible to successfully immunise growing lambs with these antigens (Vervelde et al., 2001), as might be predicted from the observation that natural immunity is slower to develop in lambs than mature sheep. Interestingly, this problem seems to have been overcome through a different choice of adjuvant, although, because whole ES was used in the trial, it was by no means clear whether protection could be attributed to the 15 and 24 kDa components alone (Vervelde et al., 2003). Unfortunately, attempts to reproduce protection with various recombinant versions of the 15 and 24 kDa molecules have not yet been successful (Vervelde et al., 2002).

In two separate trials a thiol-binding fraction of the ES from adult *O. ostertagia* reduced the egg counts of vaccinated calves by about 60%, though there was no effect on worm numbers (Geldhof et al., 2002, 2004). The same fraction, but from a detergent extract of *O. ostertagi*, was ineffective. In contrast, trials with *Haemonchus* found protection through lower egg counts and worm burdens with a detergent fraction of worm membranes, but not with a fraction extracted with saline. However, a thiol-binding fraction from *Haemonchus* ES also reduced homologous egg and worm counts by about 50% (Bakker et al., 2004).

5. Nematode and fluke gut antigens

The gut membrane approach was first applied successfully to ticks in research, which culminated in the launch of the recombinant vaccine against *Boophilus microplus*, the Australian cattle tick (Willadsen et al., 1995). The principle is straightforward. The host is immunised with appropriate gut membrane proteins from a haematophageous 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. Eventually, the parasite becomes detached 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, *H. contortus*, from which several different gut membrane proteins or protein complexes have been isolated, giving more than 80% reduction in eggs together with greater than 50% protection against worm numbers when tested under experimental conditions (Jasmer et al., 1993; Smith et al., 1994, 1997; Knox et al., 1999).

The best characterised and most effective of these are known as H11 and H-gal-GP, which, respectively, consist of a family of microsomal aminopeptidases (Smith et al., 1997), and a complex containing protective aspartyl and metallo proteases (Smith et al., 2003a,b). It is presumed that all three protease families are involved in the digestion of the blood meal, a function compromised by antibodies in vaccinated sheep. Immunisation with a combination of H11 and H-gal-GP can offer substantial protection against natural haemonchosis in grazing sheep (Smith et al., 2001a). Attempts to produce recombinant *protective* versions of these antigens has proved to be frustratingly unsuccessful to date, though attempts are still on-going (Newton and Meeusen, 2003; Smith et al., 2003a,b).

In the case of H11, recombinant enzymically active versions of three different aminopeptidase isotypes have been synthesised. Sheep immunised with a combination of these responded with serum titres of enzyme neutralising antibodies indistinguishable from animals immunised with native antigen, yet, unlike these positive controls, they were not protected against challenge. Three possible reasons for this failure were discussed (Newton and Meeusen, 2003), two of which involving glycosylation and contaminating proteins are commented on further here.

Because the recombinant aminopeptidases were derived from insect cells, they were glycosylated differently from the native nematode enzymes, a feature which Newton and Meeusen (2003) thought could be critical. While this explanation may indeed be valid, in our view the jury is still out and should remain so. After all, native Bm86 from *B. microplus* and native To45 from *T. ovis* (Lightowlers et al., 2003) are also glycoproteins, yet these remain highly protective when in recombinant form. Similarly, if glycan alone was so crucial it might be expected that preparations of native integral membrane intestinal cell glycoproteins from, e.g. *Caenorhabditis elegans*

or *Teladorsagia circumcincta* might be almost as protective for *Haemonchus* as homologous preparations, but this is not the case (Redmond et al., 2004; Smith et al., 2001b). If glycan is important for protection, its effect must be due to conformational epitopes rather than to simple sugars, else fully denatured H11 would also protect.

The second explanation raised by Newton and Meeusen (2003) was that the recombinant aminopeptidases were much less protective than native H11, because the latter contained contaminating molecules crucial for vaccine efficacy. Our view is that this possibility is just as real as the wrong glycan theory. Like many integral membrane proteins H11 is very difficult to purify completely and detecting other molecules within the type of native H11 vaccine preparations listed by Newton and Munn (1999) is not difficult. Perhaps immunisation with aminopeptidases alone is insufficient to interfere adequately with *Haemonchus* nutrient uptake, rather a combination of digestive enzymes is needed for a really effective vaccine.

Serum from sheep repeatedly infected and immune to *Haemonchus* did not recognise the parasite's integral gut membrane proteins (Smith, 1993), leading to the conclusion that, like certain *Boophilus* gut proteins, they were ''hidden'' antigens. However, more recent evidence has been provided that *Haemonchus* gut membrane proteins are recognised by vaccinated sheep or goats after a challenge infection (Andrews et al., 1995; Jasmer et al., 2003), though not to an extent where the anamnestic response is capable of conferring protective immunity (Andrews et al., 1995; Smith et al., 2001a).

A major advantage of the gut 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 (Tavernor et al., 1992; Smith, 1993), goat kids (Jasmer and McGuire, 1991) and periparturient ewes (Andrews et al., 1995) can be successfully immunised and that some protective immunity is even transferred by maternal antibody (Andrews et al., 1995). On the other hand, when circulating vaccine antibody titres have waned with time, a challenge infection does not boost the immune response sufficiently rapidly or effectively for the infection to be eliminated (Smith et al., 2001a). This is in stark contrast to conventional microbial vaccines and would seem to be a serious disadvantage because frequent immunisations might be required for an effective level of protection to be maintained. However, experimental evidence suggests otherwise. The mechanism of protection in lambs immunised by this method mainly affects late fourth stage and older worms, whereas incoming larval stages, which are not yet blood feeders are largely unaffected (Smith, 1993; Smith and Smith, 1993). Thus, when immunised 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 and 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 (Smith, 1993; Smith and Smith, 1993).

Attempts have been made to determine whether the gut antigen approach could be successfully deployed against species of nematode, which are not direct blood feeders. The precise diet of economically important genera like *Ostertagia* and *Dictyocaulus* is not known, but they contain host immunoglobulin, presumably ingested with mucus, tissue fluids and/or serous exudates (Murray and Smith, 1994). Partial success has been reported for *O. ostertagi* with homologues of H11 and H-gal-GP, the most protective *Haemonchus* antigens (Smith et al., 2000), but not for *T. circumcincta* (Smith et al., 2001b). Interestingly, antigen preparations from each of the latter species were more effective against *Haemonchus* than a homologous challenge, suggesting that the relative failure was more likely to be due to inadequate ingestion of host blood and therefore antibody, rather than to a faulty antigen preparation (Smith et al., 2000, 2001b).

The glutathione *S*-transferases (GSTs) 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 (Sexton et al., 1990). GSTs are hidden antigens because they are not recognised serologically by sheep or cattle infected by this parasite (Creaney et al., 1995). Although they are present on the surface of the gut cells of *F. hepatica*, GSTs are also distributed in the parenchema and tegument of the parasite

(Morrison et al., 1996), and so it is not clear whether the mode of protection is analogous to that described for the nematode gut membrane enzymes. GSTs are thought to be involved in the fluke's metabolism of xenobiotics, transport of anionic compounds and the detoxification of lipid peroxides.

Sheep and cattle immunised 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 (Creaney et al., 1995). The effect depended very much on the adjuvant employed, but the formulation most effective against *F. hepatica* did not work against *Fasciola gigantica* (Estuningsih et al., 1997), suggesting that it was not possible to extrapolate results from one *Fasciola* species to another. It is not clear by what mechanism protection is induced but the simplest possibility that anti-GST antibody neutralises these enzymes, seems to have been discounted. Unfortunately, an attempt to achieve protection with a recombinant GST in cattle was not successful (De Bont et al., 2003).

Two South American groups have recently made significant advances towards a vaccine for ovine liver fluke. Piacenza et al. (1999) reported almost 90% reduction in flukes following immunisation of lambs with a leucine aminopeptidase isolated from a detergent extract of adult *F. hepatica*, presumably by a mechanism akin to that described for H11, the *Haemonchus* microsomal aminopeptidase family. Even more impressively, Almeida and others found an almost complete absence of worms and liver pathology in sheep immunised with a *recombinant* version of a fatty acid binding protein derived from *S. mansoni* known as Sm14, which is also a highly effective against homologous challenge in rodent models of schistosomiasis (Almeida et al., 2003). *Fasciola* is believed to employ fatty acid binding proteins to obtain fatty acids from host blood and it is assumed that immunisation somehow blocks this mechanism with fatal consequences for the parasite. Even though the protective effect was reproduced in two separate experiments, the number of sheep in each group was small and, as the authors themselves pointed out, more realistic field trials are required to evaluate the product, which appears to be the closest thing to a commercial fluke vaccine described to date.

Compared to the abomasal species, little work has been published recently on protective antigens for small intestinal nematode parasites of ruminants. One significant discovery described a clumping mechanism mediated by mucus antibodies whereby sheep rejected *T. colubriformis* larvae. The antigen concerned, which is only found on the third larval stage and its sheath, was identified as being composed of 35 kDa carbohydrate subunits and has been termed CarLa (Harrison et al., 2003a,b). Similar molecules are present in *Nematodirus*, *Cooperia*, *Haemonchus* and *Teladorsagia*. It will be interesting to know whether sheep can be immunised with this antigen and, if so, whether they are protected. However, even if this can be achieved, the problem of mass producing this carbohydrate molecule cost effectively for a commercial vaccine will remain.

A recombinant form of a 17 kDa globin-like molecule from *T. colubriformis* (Frenkel et al., 1992) was reported to reduce egg and worm counts of young lambs by up to 50% after being given intraperitoneally in Freunds adjuvant (Emery et al., 1999). However, several other trials with the same antigen given in different adjuvants and by other routes produced mixed results (S.J. McClure, personal communication).

6. Vaccine formulation and mode of delivery

The choice of adjuvant can be crucial for the success of any vaccine. For example, using *Haemonchus* ES as antigen, much better antibody responses and protection were achieved in lambs with alhydrogel as adjuvant than with dimethyl dioctadecyl ammonium bromide (Vervelde et al., 2003). However, when alhydrogel was compared with QuilA for immunising calves with ES thiol antigen from *O. ostertagi*, only those immunised with QuilA were protected (Geldhof et al., 2004). In the case of the cestode oncosphere antigens, an adjuvant, which stimulates a high titre antibody response is clearly what is needed. With the nematode gut antigens the requirement is the same, except that the response should also be prolonged as much as possible. Because the mechanisms of protection are not yet understood, it is not yet clear what the requirements of adjuvants for the fluke antigens are, but experiments to date have

shown that the choice can be critical. For those species of nematode, which reside in the small intestine, it may be necessary for a recombinant vaccine to stimulate facets of the mucosal response for effective protection to be induced. A variety of adjuvants given by different routes have been tested and the possibility of using *Salmonella* vectors for achieving this has been pursued (Brahmbhatt et al., 1997). The concept of naked DNA vaccines is attractive particularly because it could short circuit the need for producing functional recombinant proteins. Unfortunately, immunising sheep with DNA alone was not encouraging in the case of the 45W *T. ovis* antigen, since only low titre antibody responses were invoked and no protection against challenge was observed. However, sheep primed by this naked DNA and boosted by the same gene in an adenovirus vector had much higher antibody titres and showed correspondingly high levels of protection following challenge (Rothel et al., 1997).

7. Impediments to vaccine development

In recent years, our understanding of innate and acquired immunity, and our appreciation of the complexity of host protective responses have advanced tremendously. The dearth of available parasite recombinant vaccines, however, indicates that there remain hurdles in the quest to develop functional reagents. A great number of these impediments relate directly to the antigen, such as: (1) correct configuration and post translational modification; (2) mode of administration and duration of the response; (3) cost-effective production and dissemination; (4) product stability. In addition to these, less tangible obstacles such genetic diversity and millions of years of adaptive and co-evolutionary forces among host and parasite assemblages are being seen today as formidable deterrents to achieving our goals. The section to follow briefly synopsises these issues and their roles in vaccine development.

7.1. Parasite genetic diversity

Researchers aspiring to develop nematode vaccines based on naturally acquired mechanisms of immunity have found this to be a formidable task, where numerous factors have contributed to the lack of success, such as generating a product that is broadly efficacious over a prolonged grazing season, maintaining a balance between reducing/eliminating disease pathology and preserving animal productivity, producing a vaccine that is adaptable to a multitude of management programs, and preserving sample stability and integrity (Sonstegard and Gasbarre, 2001). The genetic complexity of the organisms suggests that hundreds to thousands of antigens are potentially involved in the cascade of the infection process. Parasites undergo several well-defined life-cycle changes even within their definitive hosts, which present unique subsets of antigens for the immune system to engage at each level of development. Delineating which of these to target for recombinant vaccine development has been a daunting task and has been the subject of substantial review and discussion (Sonstegard and Gasbarre, 2001).

In association with antigenic complexity, is the potential role for both inter- and intra-population genetic and antigenic diversity in the heartiness of the species and its ability to circumvent host immune intervention. Prior to the advent of PCR, research on population diversity was limited to morphological studies and anecdotal biological data at best. However, as early as 1993 using mitochondrial DNA analysis, Dame et al. (1993) demonstrated that substantial genetic variation occurred both among and within populations of *O. ostertagi*, to the extent that 98% of the level of variation separating any two populations was partitioned among individual worms within a single population. Further studies showed that this phenomenon was not unique to *Ostertagia* but present in other trichostrongyles as well (Blouin et al., 1995). Collectively, these data suggest that responses to any given antigen may not necessarily be unilaterally protective even against individuals within the same population of worms. The plethora of ‘‘partially protective’’ recombinant antigens amidst a higher level of protection with the cognate naturally derived antigens may be due, in part, to this type of genetic diversity.

Due to population mixing among species of cattle nematodes that results from the artificially high migration rate of the host, genetic subdivision of the parasites, even within broad geographical areas is lacking. This leads one to hypothesise that other

transmissible traits including but not limited to resistance to drugs and vaccines would spread with equal speed throughout a species (Viney, 1998; Blouin et al., 1995). The rapid deployment of drug resistance among parasitic nematode groups of sheep, as well as that now being observed in trichostrongyles of cattle lends credence to such a hypothesis.

It is difficult, to ascribe the less than stellar results from field tests of recombinant vaccines to these findings only. Indeed, some would argue that the lack of genetic subdivision could simplify the identification of unilaterally effective vaccines. Yet, within other nematode genera, experiments involving heterologous challenge suggest that antigens associated with protective immunity are not necessarily conserved among species or even among isolates of the same species (reviewed in Maizels and Kurniawan-Atmadja, 2002). Consequently, it is prudent to ascertain or consider the influence of protein polymorphism among natural populations during the process of vaccine development.

7.2. Host genetic diversity

In like manner, when evaluating antigens one cannot rule out genetic variability within the host species and its relation to immunological non-responsiveness to candidate vaccines (Quakyi et al., 1989). Indeed, model predictions dating back to 1985 concluded that controlling parasitism by vaccinating herds that were genetically heterogeneous in their ability to mount protective responses would be extremely difficult (Anderson and May, 1985). To this end, evidence has been advanced demonstrating that genetics plays a role in innate or acquired resistance within a herd of nematode infected animals where Leighton et al. (1989) showed that the number of nematode eggs/gram (EPG) in feces of pastured cattle was strongly influenced by host genetics. Further, research demonstrated that high EPG values were not randomly distributed throughout the herd, but that the majority of parasite transmission was the result of a small percentage of ''highly susceptible'' animals within the herd (Genchi et al., 1989). This suggests that vaccines to reduce parasite transmission and therefore host pathogenicity may need only be targeted to the small population of susceptible hosts within a herd. Work is currently under way to identify genetic markers for delineating nematode susceptible and resistant cows (Gasbarre et al., 2001; Sonstegard and Gasbarre, 2001) and sheep (Beh et al., 2002) to assist in managing this problem, naturally; however, one can envision that further attenuation might be achieved when coupled with an appropriately designed and administered vaccine.

The contributions that animal modeling has made to our understanding of immunity and the polarisation of immune responses to infection via antigen specific responses are innumerable. Still, examples persist in large animal research demonstrating deviations from conventional wisdom, such as: (1) the unlikely down-regulation of IL-4 receptor α and IL-13 receptor α-1 transcription during acute nematode induced Th2 host responses in swine (Zarlenga et al., 2004); (2) the significantly lower levels of IL-12β2 receptor transcription in swine lymphocytes (Solano-Aguilar et al., 2002) which may influence the timing and intensity of Th1-associated responses; (3) the concomitant elevation of IFN-γ and IL-4 transcription in nematode (Canals et al., 1997) and virus (Waldvogel et al., 2000) infected cows; (4) the near absence of rapid expulsion of adult worms in large animals and man (Behnke et al., 1992). Although small animal modeling has shown quite convincingly that mechanisms involved in antigen recognition are generally conserved among a broad spectrum of hosts, it cannot be assumed that specific parasite immune evasion mechanisms are congruent across model and natural host systems. Model systems may not accurately mimic the parasite's natural environment or the inter-workings of the host–parasite relationship. Thus, problems surface in the expectation that successful vaccination of outbred, natural host populations can be predicated upon studies with inbred, genetically matched animals. Literature is teeming with laudable results from parasite vaccination trials performed in rodents only to be ensued by lackluster data from studies conducted in large animals. It is unfortunate as well to consider that many candidate antigens have been cast aside over the years because of negative results obtained in rodent models.

The use of host-adapted, laboratory strains of parasites in research is a well established practice but not without its problems when in use for vaccine development. Work by Goyal and Wakelin (1993) showed significant variation in host responses to

Trichinella infections in high-responder NIH mice relative to low-responder C57BL/10 mice. Thus, the choice of laboratory host can skew our understanding of protective responses. It has been known that laboratory strains of rodents can actually select subpopulations of organisms. Such was the case with *Taenia crassiceps* where research showed that after years of propagation of a fox isolate in laboratory mice, not only did the parasite lose its ability to propagate in the natural host, but obvious alterations in its morphology and antigenic character had also occurred (Freeman, 1962; Fox et al., 1971). In *S. mansoni*, polymorphic loci originally found in a baboon isolate underwent host-induced selection and became fixed with time after propagation in mice (LoVerde et al., 1985). Surprisingly, during these same studies it was determined that allele-a of phosphoglucose isomerase became fixed in the murine system in as little as a single generation. With this in mind, Minchella (1985) proposed that in a parasite–host assemblage, host imposed selective pressures force the parasite to make a genetic commitment to co-evolution.

7.3. Evolution and vaccines

The process of evolution is among the most difficult and elusive forces scientists must contend with in developing parasite vaccines. This problem is exacerbated with time where co-evolution between the host and parasite results in the selection of shared systems of host adaptation and immune evasion, and thus a microenvironment suitable for the longevity of the parasite. In some instances, the correlation between definitive and intermediate hosts may involve very recent evolutionary events, as is the case with human taeniids, where the presence of the parasites in the intermediate hosts (bovine and swine) was likely a consequence of their recent domestication by man rather than by vertical transmission from the intermediate host (Hoberg et al., 2001) to hominids. Thus, in this example, the absence of a well adapted host–parasite assemblage is one probable impetus for rapid calcification of the taeniid muscle cysts in their intermediate hosts, i.e. the host already does a good job of developing protective immunity. As a result, one might predict a greater success in achieving near 100% immunity when vaccinating an animal that already has a strong natural immunity against infection (Lightowlers et al., 2003) than in those situations where the host–parasite relationship has had sufficient time to evolve. The terminal localisation of taeniid cysts amidst non-mucosal surfaces, a character not shared by intestinal parasites of grazing animals, could also contribute to the extraordinarily high levels of natural and vaccine-induced protection.

Parasites have developed unique strategies to obviate immune intervention by the host, such as: (1) coating themselves in host proteins so as not to be recognised as foreign; (2) continually producing novel surface proteins, impairing the effectiveness of the host's defense mechanisms; (3) generating components that down-regulate host protective responses; (4) ''mimicking'', or producing proteins that simulate host proteins; and in rare cases; (5) intracellular localisation as is the case with *Trichinella*. Because of these and many other avenues of parasite survival, researchers must contend with the existence of imperfect (partially effective) rather than sterile protection, and in so doing the evolution of vaccine resistance and its relation to virulence. In this regard, several hypotheses have emerged. One theory suggests that artificial intervention against parasites can be classified in two broad categories; those methods that reduce pathogen growth rate, and those that are infection-blocking and reduce transmission (Gandon et al., 2001). In this model, eradication based upon reducing within-host growth rate will select for higher virulence whereas those based upon reducing the probability of infection will select against virulence and possibly lead to eradication (Gandon et al., 2001, 2003). This theory arises from the trade-off model (Anderson and May, 1982; Ewald, 1983), which proposes an association between parasite transmission and host survival or well-being. Still, there are others advocating that direct selection against virulence and not virulence management might be a better approach (Ebert and Bull, 2003). Both concepts assess the evolution of virulence generically with respect to the infectious agent and to this end concede that neither is a panacea for addressing the problem. In developing recombinant vaccines against nematodes, however, transmission potential has often been directly equated with virulence. Collectively, both modeling and current data raise the question as to how good do vaccines have to be to effect the desired results.

8. How good do worm vaccines have to be?

Before embarking on the arduous task of developing vaccines against any disease agent, it is prudent to define an achievable endpoint. In the case of nematodes of ruminants it maybe more appropriate to consider a vaccine as an epidemiological tool to maintain low level pasture contamination, rather than as a weapon to abolish infection completely. Barnes et al. (1995) developed a mathematical model for simulating *Trichostrongylus* populations in grazing sheep and compared 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; and if the vaccine was based on a hidden antigen, then protection of 80% of the flock with 80% efficacy would give better control than a conventional anthelmintic programme.

On a similar vein, it has been advocated that a vaccine which can reduce the *O. ostertagi* egg output of first season calves by 60% for the first 2 months after turn-out, would be sufficient to control bovine ostertagiosis under North Western European conditions. This conclusion was based on: (1) the bulk of the pasture contamination in the peak mid summer months is derived from this early contamination; (2) because exposed calves rapidly acquire an immunity which controls *Ostertagia* fecundity over the peak period; (3) because effective disease control was obtained by an anthelmintic preparation of similar efficacy (Geldhof et al., 2002, 2004).

Approaches to nematode vaccine development have focused substantially on altering the host's immune response to the presence of the organism. In this way, a successful vaccine historically has been measured by reducing or eliminating egg output (transmission potential) or worm burdens (growth-rate). Today, however, it may be prudent to consider alternatives to conventional thinking and appraise the level of disease rather than the level of parasitism when determining the efficacy of a vaccine. Such an approach would include immunizing against pathogenic factors or effects rather than against the organism itself thereby allowing host-specific antibodies to mitigate the pathology associated with the infection. This method was evaluated in developing a vaccine against *Trypanosoma congolense* in cattle (Authie et al., 2001). Animals immunised with two cysteine proteinases and subsequently challenged with *T. congolense* showed no substantial effect on the establishment of infection nor in the development of acute anaemia; however, immunised cattle maintained or gained weight during infection and showed conversion to more normal haematocrit levels after only 2–3 months of infection. Some would argue that this might generate unexpected results, such as the evolution towards higher virulence (Gandon et al., 2001); however, even in the best models, virulence evolution can be either positive or negative depending on the organism's life-history and host's heterogeneities. In the end, where model systems seem to target either transmission or virulence as the best measures for control, a vaccine directed at both maybe required for the optimal well-being of the animal.

Alternative mechanisms by, which worm fecundity and/or survival become attenuated within the host have been proposed (Viney, 2002). Customary thinking owes this phenomenon to direct immune intervention by the host; however, little if any consideration has been given to indirect attributes, such as the energy expended by the organism to abate or counteract the host immune response during survival. Thus, there is a cost to the worm in terms of self-protection and repairing damage caused by the host that must be overcome in order to survive. Accepting such a premise, Viney (2002) suggests that new targets for immunisation maybe obtained by differentially evaluating transcription or protein patterns from parasites subjected to extreme immune pressure against those from parasites in naive animals and under minimal immune pressure. Any observed changes could be ascribed to the organisms efforts to mitigate the effects of the stronger immune response in the challenged animals.

9. Conclusions

Tremendous advances towards worm vaccines for grazing ruminants have been made in the last decade. Several highly protective antigens have been discovered, the genes encoding several of these have been cloned and, in the case of several cestodes at least, protective recombinant antigens have been

synthesised. The application of micorarrays, real-time PCR, RNA interference and proteomics have substantially advanced gene discovery, but as the era of genome sequencing winds down, scientists must turn their attentions to linking genomic and proteomic data to the parasitic nature of an organism in order to target genes likely involved in host recognition, infection, and immune evasion. Aside from advances in the art of gene discovery, however, there remain considerable hurdles to be overcome before monovalent vaccines for GI nematodes reach the market place, where the prospects for multivalent nematode vaccines are more distant. Specific obstacles include the production of stable, cost-effective recombinant versions of the protective antigens with the correct tertiary configuration to elicit effective and prolonged responses, and identifying appropriate adjuvants for administering the vaccines. Nonetheless, if the rate of progress in vaccine development seen in the last 15 years can be maintained in the years to come, and these advancements can be parallelled by our understanding of parasitism, it is reasonable to consider that the introduction of a nematode vaccine will be in reach before long.

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