Overview

Parasite vaccines – recent progress and problems associated with their development

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INTRODUCTION

The treatment and prevention of parasitism in both humans and livestock continues to rely almost exclusively on the use of antiparasitic drugs – an approach which has limitations, particularly as re-infection, which occurs rapidly in endemic regions, is not prevented. In addition, the widespread appearance of drug-resistant parasites of animals (Kaplan, 2004;) together with emerging evidence of resistance problems in human parasites (Fallon *et al*. 1995; Ismail *et al*. 1996; De Clerq *et al*. 1997; East African Network for Monitoring Antimalarial Treatment, 2003), emphasise the importance of developing alternative methods of control, with anti-parasite vaccines a prime target.

The advent of recombinant DNA technology in the 1980s and the ability to produce recombinant parasite proteins (Ellis *et al*. 1983; Kemp *et al*. 1983) was heralded as a major breakthrough which would make the development of parasite vaccines a probability. However, some 25 years on, and despite further progress in our understanding of the immune effector mechanisms involved in generating host-protective responses to parasitic infections coupled with recent technological advances in genomics and proteomics, only a few recombinant vaccines against parasitic diseases of livestock have reached the point of being marketed. The first recombinant vaccine against a human parasite continues to remain elusive.

This supplement aims to summarise recent progress in the development of vaccines against some of the major human and veterinary parasites. Apologies to readers whose particular parasite of interest is not included but space does not permit a fully comprehensive coverage of advances in every parasite. The supplement aims to highlight some of the specific and generic problems/challenges scientists working in this field of research must face and overcome in order to achieve success. It brings together the thoughts of leading researchers in protozoan, metazoan and ectoparasite biology and highlights the commonality of approaches used as well as many common constraints to progress.

A fundamental requirement in vaccine development is to define the level of protection that is required to achieve control, be this using a vaccine alone or as part of an integrated package of control measures. The parameters used to define control requirements may vary from achieving sterile immunity, through reducing the impact of infection to blocking transmission. In general terms, the 'performance' requirements for a vaccine are defined on the basis of epidemiological data and mathematical modeling. In veterinary parasitology, the livestock producer will inevitably compare a vaccine with control achieved with anthelmintic drugs and ectoparasiticides, which, when first introduced, approach 100% efficacy. However, it is unlikely that anti-parasite vaccines will attain this level of efficacy and computer modeling of population dynamics suggest it is not essential (Barnes, Dobson and Barger, 1995; Williams *et al*. 2002).

THE PROTECTIVE IMMUNE RESPONSE

Considerable progress has been made in identifying antigens which induce protective immunity in all the major parasites targeted. However, it is at least 20 years since the ability to produce recombinant parasite proteins was first reported and progress towards producing effective recombinant sub-unit vaccines is frustratingly slow. Recombinant proteins with the required efficacy are rare, spectacular exceptions being vaccine developments in ticks and cestodes, these developments constituting

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land-mark achievements (see reviews in this supplement by Willadsen and Lightowlers). There are numerous possible explanations for this outcome, not least of which is the complexity of the protective immune response. Wynn and Hoffmann (2000) noted that successful vaccine development for schistosomiasis had been hindered by a lack of consensus on the type of immune response required and an incomplete knowledge of the effector mechanisms which mediate immunity. The same comment can almost certainly be applied to all parasites for which vaccines are sought and is a recurring theme in the reviews in the present supplement. The maintenance of natural immunity is often dependent on repeated infection, may be stage specific and will be dependent on different antibody classes and T cell responses.

ATTENUATED LIVE VACCINES

Current evidence suggests that stimulating a combination of these protective responses would be required for effective vaccination and this ambition has been addressed by the development of live vaccines, most notably for the control of coccidial infections in poultry (Innes and Vermeulen in this supplement). Infection is self-limiting but induces protective immunity where the pathological consequences of infection are minimised by the use of attenuated organisms or the use of avirulent parasite strains. This remains the singularly most successful approach to vaccination. Attenuation by irradiation has resulted in the development of anti-nematode vaccines for the control of lungworm (*Dictyocaulus*) in cattle (Jarrett *et al*. 1958) and sheep (Sharma, Bhat and Dhar, 1981) and for hookworm (*Ancylostoma caninum*) infections in dogs (Miller, 1978). However, the absence of *in vitro* parasite culture systems renders these vaccines costly to produce and introduces problems associated with batch-to-batch consistency. In addition, they tend to be unstable and have a relatively short shelf-life. In the case of protozoal infections, most vaccines are live vaccines which result in a low-level infection in the host and stimulate protective immune responses mimicking natural infections (Innes and Vermeulen in this supplement; Cornelissen and Schetters, 1996). This has been achieved by the use of parasite strains that have been selected for: (1) Complete, but shortened life cycles; (2) Truncated life cycles and; (3) Virulence attenuated by repeated passage. Other kinds of live vaccines are low-dose infections, particularly in situations where the infection is transient (e.g. coccidiosis infections in chickens), and the use of chemotherapeutically controlled infection (e.g. *Theileria parva*; Morrison and McKeever in this supplement).

Humans and animals can be protected to varying degrees against one of the causative organisms of malaria (*Plasmodium falciparum*) by immunisation with radiation-attenuated sporozoites (Hoffman *et al*. 2002) and this approach is undergoing serious development (Richie in this supplement). Whole parasite approaches to vaccine manufacture have been considered impractical because of manufacturing difficulties and fears that breakthrough infections may occur (Good, 2005). A very recent report (Mueller *et al*. 2005) has reawakened interest in whole parasite approaches. These authors used reverse genetics to show that one gene, UIS3 (up-regulated in infective sporozoite gene 3), is essential for early liver stage development. UIS3-deficient sporozoites infected hepatocytes but were unable to establish blood-stage infections *in vivo*, and did not lead to disease. Immunisation with UIS3-deficient sporozoites completely protected mice in a sustained and stage-specific manner against infectious sporozoite challenge.

Vaccination against human cutaneous leishmaniasis by inoculation of virulent organisms from the pus of an active lesion is a long-standing practice (Kedzierski *et al*. in this supplement). This process, known as 'leishmanization', is still used in some countries, notably Uzbekistan. In addition, promastigotes of *L. major* grown in culture induced protection against natural infection. However, there are several basic, logistical problems which preclude the widespread use of this procedure to prevent cutaneous leishmaniasis, including difficulty in standardising the virulence of the vaccine and occasional severe, persistent lesions resulting from the inoculum (Nadim *et al*. 1983).

CRYPTIC EPITOPES/ANTIGENS

This approach avoids reliance on antigens or epitopes that are immunodominant following natural infection. In the case of malaria (see review by Richie in this supplement), the immunodominant antigens are often highly polymorphic with the polymorphic determinants being the focus of adaptive immune responses (Good *et al*. 2004). Cryptic antigens and epitopes are not immunogenic or are weakly immunogenic, during natural infection but, when presented to the immune system in an appropriate manner, can induce protective immune responses. Cryptic antigens have been sought and tested with good effect in veterinary parasitology. Here, the antigens are often referred to as 'hidden' or 'concealed' and are not part of the normal host-parasite interaction and do not normally stimulate a protective immunological response (Willadsen in this supplement). Typically, these antigens are located in the gut and, when antibody to the antigen is raised by vaccination, it is ingested when the parasite feeds, binds to the surface of the gut and damages the parasite. The approach appears most applicable to blood-feeding parasites such as ticks

(Willadsen in this supplement) and nematodes (Bethony *et al*. in this supplement) and the antigens are often enzymes implicated in blood meal digestion. The downside of the 'hidden' antigen approach is that the antibody response is unlikely to be boosted by natural parasite exposure and a need for frequent booster vaccinations might be anticipated.

ANIMAL MODELS

In humans, parasite vaccine development is highly dependent on the use of animal models, firstly to define the immune responses which may mediate protection and, secondly, to evaluate the protective efficacy (and safety) of candidate antigens prior to clinical trials. In the particular case of amoebiasis (Stanley in this supplement) there is an urgent need for a non-human primate infection model to allow vaccine testing. How appropriate are infection models? Candidate antigens are usually evaluated in animal models and often show reduced efficacy when tested in the natural host. The development of vaccines against veterinary parasites has the advantage that trials can be conducted in the natural host/parasite system. This use of animal models is complicated by the fact that the parasite may not show quite the same infection characteristics as in the natural host. Rodent models are in routine use yet the data can be contradictory and potentially misleading. For malaria, there are some 5000 candidate vaccine proteins but assessing their importance is hampered by a lack of validated models that will reliably predict performance in humans (Richie and Saul, 2002). Wyn and Hoffmann (2000) noted that studies of *S. mansoni* in rats and humans indicated that a vaccine which exploits Th 2-based effector mechanisms may be most appropriate (Capron and Capron, 1994) while vaccination studies in mice indicated that T cell-mediated responses involving IFN-γ and IL-12-dependent mechanisms might be required (Wilson and Coulson, 1999). Recent work in double cytokine-deficient mice indicated that highly polarized Th1- and Th2-type cytokine and antibody responses contribute equally to vaccine-induced immunity to *S. mansoni* (Hoffmann *et al*. 1999). In hookworms, hamster and dog L3 challenge systems are now being used routinely for preclinical vaccine development. Hamsters infected with *A. ceylanicum* experience weight and blood loss which is similar to heavy infestations in humans (Bungiro *et al*. 2001). On the downside, blood loss is not a common symptom of *A. ceylanicum* infestation in humans indicating that disease pathogenesis differs between the natural and laboratory host, differences which may lead to misleading predictions of vaccine efficacy in the target host-parasite system.

CONTINUED ANTIGEN DISCOVERY – EXPLOITING EST/GENOMES, SIGNAL SEQUENCE TRAP, RNAINTERFERENCE (RNAI)

The recent advancements in parasite genome sequencing projects and expressed sequence tag (EST) analyses provide an alternative, invaluable source for identifying further potential vaccine antigens through logical mining of databases *in silico*. For example, a recent query of the *Plasmodium* genome database (PlasmoDB) searching with the following criteria: (1) Secreted proteins; (2) Conservation between *Plasmodium* species, but not in the human genome; and (3) Abundancy in the late schizogony stage, has yielded a significant number of novel and potentially important vaccine candidates which will merit further investigation (Tongren *et al*. 2004). Genome and EST sequence datasets can be mined for potential vaccine candidates by the use of algorithms to identify NH_2-terminal signal peptides which are found on secreted proteins and on type I membrane (surface) proteins (McManus and Dalton in this supplement; Dalton *et al*. 2003). Another approach is the use model organisms to identify potential key genes, for example the use of the free-living nematode *Caenorhabditis elegans* as a model for parasitic nematode infections. With the full genome available, considerable effort is being made to define gene function using traditional reverse genetics approaches and RNAi. Many of the genes have close homologues in parasitic nematodes and the hope is gene knockdown which results in a lethal phenotype would point at parasite homologues which may be useful vaccine targets. The development of RNAi in parasitic helminths (reviewed by Britton and Murray, 2006) provides the possibility of defining if a vaccine target is critical for parasite survival by specific gene knockdown.

The technology required to identify, clone and express genes, genome sequencing, array technologies etc. have all come on stream since the 1980s. It is worth noting a comment by Pearce (2003) with reference to schistosomiasis, where he states that ''compared with during the 1980s, the last decade has seen the discovery of few new vaccine candidates''.

IMPROVING THE DEGREE OF PROTECTION – ANTIGEN COMBINATIONS

The efficacy achieved by vaccination often falls short of that required to control the disease. Should we focus more on testing antigen cocktails? After all, it is clear that parasites are complex organisms and it is probably naïve to think that a protective immune response could be stimulated by vaccination with a single protein. For example, vaccination of cattle with a combination of two recombinant oncosphere proteins of *T. saginata* induced almost complete protection against experimental challenge infection

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whereas, when used individually, neither antigen was protective (Lightowlers, Rolfe and Gauci, 1996). Also in cattle, vaccination trials conducted with purified, native *F. hepatica* antigens, a combination of cathepsin L1 and haemoglobin gave significantly higher levels of protection compared to animals immunised with either antigen alone (Dalton *et al*. 1996) while recombinant antigens given in combination were shown to increase vaccine efficacy against both the cattle tick *B. microplus* (Willadsen *et al*. 1996) and hookworm (Mendez *et al*. 2005). DNA vaccination offers the possibility of developing multivalent vaccines by fusing genes together in a single construct, an approach being tested for *Leishmania* (Mendez *et al*. 2001) and malaria (Ferry, 2000; Doolan and Hoffman, 2002), these targeting more than one parasite stage.

RECOMBINANT PROTEIN PRODUCTION

The production of any successful vaccine will depend on the technical feasibility of antigen production and its formulation in acceptable adjuvants. The ease and frequency of delivery and stability are factors that will need to be considered in order to produce what must, ultimately, constitute a cost-effective, affordable product. Recombinant protein production is discussed by several contributors to this supplement but it is worthwhile highlighting some general points here.

A wide variety of pro- and eukaryotic vectors are widely available for expression of recombinant proteins and their advantages and disadvantages are discussed in Dalton *et al*. (2003). Which system do you choose when conformation and glycosylation may contribute to protection? Bacteria do not glycosylate proteins while the nature of yeast and insect cell glycan may be inappropriate. The major humoral response to *S. mansoni*-infected animals is directed towards carbohydrate epitopes (Richter, Incani and Harn, 1996; Wuhrer *et al*. 2000). Recently, a monoclonal antibody directed to one of these antigens has been shown to effectively kill schistosomula *in vitro* in the presence of complement (Nyame *et al*. 2003). However, where parasite-specific patterns of glycosylation are identified, e.g. *H. contortus* H11 (Haslam *et al*. 1996), then the currently available commercial eukaryotic expression systems will not result in appropriate glycosylation of recombinant antigens. Recent work has explored the possibility of expressing parasitic nematode-derived genes in the free-living nematode *Caenorhabditis elegans* (see Britton and Murray, 2006) to overcome these difficulties. However, in terms of vaccine development, correct post-translational processing may prove hard to repeat reliably, may introduce problems of batch to batch consistency and will increase production costs, reduce margins and are likely to make production of the vaccine commercially unviable.

ANTIGEN DELIVERY AND ADJUVANTS

The method of antigen delivery can affect the quality of the immune response elicited and vaccine efficacy. Vaccination with 'hidden' antigens (e.g. *Boophilus* and *Haemonchus*) relies on the generation of a high humoral antibody response and ingestion of these antibodies into the parasite gut with the host blood-meal. Therefore, formulation in adjuvants such as conventional oil-based adjuvants or with the saponin derivative QuilA, both of which result in a high antibody titre, is demonstrably appropriate for these antigens. Willadsen (2004) noted that extensive adjuvant trials in Australia with more than 40 adjuvant formulations in cattle and at least 50 in animal models did not identify anything superior to conventional oil formulation. Co-administration of cytokines with antigens is one approach which may be useful where protection is mediated by particular cell mediated immune responses (Lofthouse *et al*. 1996). Co-administration of interleukin (IL)-12 has been shown to enhance protective immunity induced by recombinant Sm14 in mice eliciting higher levels of TNF-alpha and interferon (IFN)-gamma, both of which were demonstrated to be important in protection through the use of knockout mice (Fonseca *et al*. 2004). Enhancement of the host immune response in chickens to vaccination with the *Eimeria tenella* MIC2 antigen was achieved by co-injection with the chicken IL-2 gene (Lillehoj *et al*. 2005; See Innes and Vermeulen in this supplement). Immune modulators such as pertussis toxin, which can induce strong IgE and IgG1 antibody types in mice (Sekiya, 1983), or cholera toxin, a potent mucosal adjuvant in mice and humans (Holmgren, Lycke and Czerkinsky, 1993) have been applied with some effect. In some instances, vaccine efficacy is likely to depend on the route of immunisation.

DNA-based vaccines have been explored extensively over the last decade. They have potential advantages not least of which is potential cost of production and vaccine stability, an important consideration when the final product is often likely to be used in sub-tropical and tropical regions where cold storage facilities will be at a premium. In addition, they may prove a good vehicle for multivalent vaccine formulations. They are particularly effective for inducing cell mediated immunity and have been tested in a number of protozoal infections including *Leishmania*, malaria and *Eimeria* (Kedzierski *et al*., Richie and Innes and Vermeulen in this supplement). One of the most promising methods of inducing strong cell mediated immunity is the heterologous prime-boost approach, in which the identical antigen is delivered sequentially

utilizing different vaccine platforms (Richie in this supplement). Progress with DNA vaccines, e.g. in malaria, has been rapid (summarised in Richie and Saul, 2002) with protection being induced in mice and monkeys, and cytotoxic T lymphocyte responses induced in humans, although antibody induction in humans is poor. Immunogenicity can be enhanced by improving expression levels with manipulations such as replacing the native gene with synthetic genes, with codon usage modified according to the target host, or by adding sequences to optimise uptake by dendritic cells (You *et al*. 2001). Plasmid delivery can be enhanced by particle-mediated gene transfer using a gene gun. Gene gun delivery of *P. berghei* CSP DNA vaccine was found to induce strong Th2-type immune responses in mice (Weiss *et al*. 2000). Another approach with great potential is to prime specific immune responses using DNA plasmids and then boost these by administration of virus constructs or recombinant proteins.

BRINGING VACCINES TO THE MARKET

Even when a vaccine with the required efficacy is indicated during the laboratory phase and in control field trials, a number of hurdles remain – in fact progression to the market place has only just begun! The reader's attention is drawn to accounts of this process in the livestock sector by Buxton and Innes (1995); Rickard *et al*. (1995) and Willadsen *et al*. (1995) and some of the issues are addressed in this supplement. The issues are numerous. Hotez *et al*. (2003) listed 8 'obstacles' which would need to be addressed before a hookworm vaccine could become a reality. From an immunological standpoint, issues such as hookworm-induced immunosuppression, the effects of co-infection with other helminths, cross-reactivity to autoantigens and toxicity of the protective response all require attention. Then there is the issue of who pays given that hookworm is a disease of the 'poorest of the poor' and, hence, there would be no commercial market for a vaccine. Richie and Saul (2002) considered similar issues in the development of a malaria vaccine. They differentiated between the requirements needed to test anti-morbidity vaccines designed for use in children in endemic areas and anti-infection vaccines designed for malaria-naïve travellers and residents of low endemic regions. In the case of the former, the use of children increases the complexity of trial design and ethical considerations influence decisions about the end point for the trial. With the latter, efficacy requirements are likely to be more stringent, but simpler to test, as robust challenge systems are available for both the pre-erythrocyte and blood-stages of the parasite (Richie and Saul, 2002). Coming back to the issue of 'who pays', the vast majority of the market for anti-parasite vaccines in humans will be in developing countries, making vaccine production a financially unattractive endeavour for the commercial sector. Therefore, production is more likely to be carried out in endemic countries, possibly funded by local governments but, more likely in the early stages, by international agencies and initiatives funded by philanthropists.

In the case of anti-parasite vaccines for the livestock and companion animal sectors, vaccine development and production is likely to be subject to the commercial pressures of the market place. The simplest definition of performance requirement for a vaccine in these sectors is likely to be that it must be the equal of any existing chemotherapeutic. Therefore, it can be argued that anti-nematode vaccines in the livestock sector will have to be multivalent and the cost will not be significantly higher than existing methods of control. Of course, this 'commercial' view is modified by public concerns over chemical residues in livestock products, the development of drug resistance and the fact that some parasites become resistant to the available drugs more rapidly than others, thus creating niche markets for alternative control measures which will include vaccines.

Anti-parasite vaccines are unlikely to induce sterile immunity presenting the problem of convincing the users that such vaccines will be effective in controlling disease. Correct marketing and education will, therefore, be necessary to attain maximal uptake of parasite vaccines and to ensure their continued use in order to achieve the long-term benefits of reducing the incidence of clinical disease.

FUNDING

The most important, and fundamental, obstacle which impedes the development of anti-parasite vaccines is the lack of adequate and continued funding, an issue highlighted in almost all the contributions to this supplement. In particular, the low cost-benefits of developing vaccines against parasitic diseases of importance in developing countries are unattractive to commercial companies while, in the livestock sector, very few commercial companies are prepared to invest in the research and development stages of vaccine production. Instead these research programmes must rely exclusively on government and private sector funding. Colley, LoVerde and Savioli (2001) pointed out that there has been a serious erosion of financial support in medical helminthology research in terms of grant money awarded, the number of grants funded and investment in training. This has resulted in a consequential decrease in the expertise base and in the number of trainees interested in this field of research in general. Pearce (2003) further highlighted problems associated with the increasing difficulty of publishing partially protective experimental vaccine results in high impact journals and the consequential

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implications on the career development of young scientists. For these reasons, many research leaders are reluctant to place students or post-doctoral scientists on vaccine research programmes. Continued support for research in basic parasite biology, further understanding of host-parasite interactions and the underlying mechanisms of host evasion is imperative for informed vaccine design. This work should go a long way towards ensuring that the exciting developments in immunology, genomics and proteomics can be exploited to their full potential expediently in the development of marketable anti-parasite vaccines.

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