

Radiation-attenuated schistosome vaccination – a brief historical perspective

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SUMMARY

The high level of protection which can be induced by vaccination of a range of hosts, from rodents to primates, with live radiation-attenuated schistosome larvae offers great promise for development of a human schistosome vaccine. Studies of the irradiated vaccine models benefitted from significant funding during the 1970–90s and much was learned concerning the inducers, targets and mechanisms of immunity. Less progress was made in definition of the protective antigens involved. The application of new techniques for identifying membrane and secreted antigens has recently provided new vaccine candidates and a new impetus for schistosome vaccine development. This article is intended as an overview of some of the main lessons learned from the studies of the irradiated vaccines as a backdrop to renewed interest in schistosome vaccine development.

Key words: schistosomiasis, radiation attenuation, membrane antigens, secreted antigens, vaccine development.

INTRODUCTION

Schistosomiasis has been eradicated from a number of areas of the world through valiant integrated control measures based on snail control, drug treatment and environmental modification. In many other endemic areas morbidity has been dramatically reduced in recent times by treatment with praziquantel, the only drug now in widespread use (Fenwick and Webster, 2006). In such areas the snail host populations remain unaffected, transmission persists and reinfection is common. Relaxation of vigilance in 'eradicated' areas or of control efforts in endemic areas or development of resistance to praziquantel would rapidly lead to resurgence of the disease. Despite the potential of regular chemotherapy to control morbidity, the desirability of a vaccine to supplement and sustain chemotherapy-based control efforts remains as high as in 1970–1990 (Taylor, 1994) when schistosome vaccine research was reasonably well funded. A particular focus of attention during this time was on vaccination with radiation-attenuated larval infections which were demonstrated to be highly effective in many experimental hosts (Taylor, 1994) and this encouraged belief in the feasibility of developing a defined schistosome vaccine for humans. Further studies of irradiated vaccines yielded considerable understanding of the schistosome life-cycle stages which are the optimal inducers and targets of such immunity but limited progress was made

into defining and validating the protective antigens involved. Other, parallel approaches to vaccine development yielded several recombinant-derived schistosome proteins which showed promise in their labs of origin but less so when tested independently (Bergquist and Colley, 1998). With this, the impetus for vaccine development waned, particularly since there was a general perception in the public health community at this time that the schistosomiasis problem could be 'solved' globally using population-scale praziquantel treatment.

However, there is now a growing appreciation of the limitations of what chemotherapy campaigns alone can achieve, and recent application of proteomic and genomic techniques has facilitated the identification and characterization of membrane and secreted antigens, likely candidates for the protection induced by irradiated vaccines. So it is timely to review some of the lessons learned from the irradiated vaccine work in the hope of encouraging the new generation of those who share the belief that schistosome vaccines are both feasible and needed (Bergquist *et al.* 2005; Bethony *et al.* 2008). More exhaustive reviews of irradiated vaccines (Coulson, 1997; Dean, 1983) and of approaches to schistosome vaccine development (e.g. Hewitson *et al.* 2005; Wilson and Coulson, 2006; McManus and Loukas, 2008) are available.

IRRADIATED VACCINES – ESTABLISHMENT OF EFFECTIVE PROTOCOLS

Use of radiation-attenuated helminth infections to induce protection was first tested in the 1950s and,

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notably, led to development of the commercial live vaccine Dictol[®] against *Dictyocaulus viviparus*, a nematode of cattle (Jarrett *et al.* 1958). The vaccine comprised oral infection with larvae irradiated with 40 krad (kilorads) such that they did not reach the adult pathogenic stage but survived long enough to stimulate cells in the mesenteric lymph nodes. Prompted by this success irradiated vaccines against other helminths were soon being tested e.g. Miller (1978). In the case of schistosomes, doses of 3–4 krad of X- or γ -radiation were shown to prevent developing schistosome worms surviving to the adult pathogenic egg-laying stage while doses of 1–3 krad resulted in persistence of a small proportion of stunted and generally sterile adults (Smithers, 1962). There followed various protection studies against *S. mansoni* and *S. japonicum* in mice and repeatedly exposed rhesus monkeys using cercariae exposed to relatively low levels of radiation (Hsu *et al.* 1962; Radke and Sadun, 1963; Sadun *et al.* 1964; Smithers, 1962; Vilella *et al.* 1961) and most studies reported higher levels of partial protection to challenge with very low doses of radiation which allowed a small proportion of stunted worms to survive. These studies were carried out at a time when the prevailing notion, based on studies in rhesus monkeys harbouring unattenuated *S. mansoni* infections (Smithers and Terry, 1965, 1967), was that the adult worm is the prime stimulus to protection and that the schistosomula stages contribute little to the development of resistance. Thus, with respect to these early irradiated infections Smithers (1976) suggested that ‘... the immunizing effect is predominantly due to the few stunted worms which survive the lethal effects of radiation’. However, Hsu *et al.* (1969), wishing to avoid inflammatory foci in the lung and liver due to death of immunizing larvae in these organs, tested highly irradiated (24 or 48 krad) cercariae of *S. mansoni* and *S. japonicum* which would arrest in the lung/skin. Both radiation doses resulted in high levels of protection in rhesus monkeys (>80% fewer worms than controls) especially after repeated (>3) infections.

In the early 1970s studies were initiated using the schistosome species of domestic animals, *S. matthei* and *S. bovis*, which meant that vaccination efficacy could be assessed in natural hosts i.e. sheep and cattle. Initial studies in sheep using 1 or 2 exposures to *S. matthei* larvae irradiated at 3 or 6 krad were ineffective but high levels of partial protection >70% could be induced following 4 vaccinations and comparable protection could be induced with percutaneously applied cercariae and intramuscularly injected *in vitro*-transformed schistosomula (Taylor *et al.* 1976b). Similar protection could be induced against *S. bovis* in cattle with a single vaccination (Bushara *et al.* 1978). These studies culminated in a field trial in cattle of an *S. bovis* vaccine comprising a single intramuscular injection of schistosomula

irradiated at 3 krad (Majid *et al.* 1980). High partial protection to natural exposure was demonstrated and was followed by similar successful field testing of an irradiated, cryopreserved, schistosomula vaccine for *S. japonicum* in cattle and buffaloes, key hosts of this zoonotic schistosome species (Hsu *et al.* 1984).

In the case of the human schistosome species, *S. mansoni*, a similar approach was applied using baboons, regarded as a suitable model for human schistosomes since these primates, unlike rhesus monkeys, are natural hosts of both *S. mansoni* and *S. haematobium*. However, repeated exposure to larvae given low doses of radiation did not induce significant protection (Taylor *et al.* 1976a). This failure prompted studies in mice and rats aimed at defining the key parameters for optimal vaccination protocols. It was soon established that for resistance induced by a single exposure of mice the dose of radiation had a significant effect. Both low doses of radiation, allowing parasite survival through to stunted adult worms, and very high doses, arresting the larvae in the skin, were poorly effective whereas significantly higher protection could be induced with optimal doses (56 krad (Minard *et al.* 1978a) or 20 krad (Bickle *et al.* 1979c)). In both cases the effects of these optimal doses of radiation on the larvae were similar, resulting in a slight delay in the skin but eventual migration to and death in the lungs (Minard *et al.* 1978b; Bickle *et al.* 1979b; Mastin *et al.* 1983; Mangold and Dean, 1984). The reasons for the numerical difference in these optimal doses is not clear but since at this early time most subsequent studies in the USA, although not all (Reynolds and Harn, 1992), employed doses around 50 krad and in the UK, 20 krad. In rats, 20 krad also arrested *S. mansoni* larvae in the lungs and similarly proved to be an optimal dose (Ford *et al.* 1984a).

The superior protective efficacy of highly irradiated cercariae in mice led to renewed testing in baboons and, similarly, somewhat greater protection could be induced with optimally-irradiated infections. Thus, studies using *S. haematobium* (Sturrock *et al.* 1980; Webbe *et al.* 1982; Harrison *et al.* 1990) demonstrated high levels of protection (~90%) with multiple (2–3) exposures to 20 krad cercariae whilst lower protection was obtained with 3 krad or 60 krad and significantly less with a single vaccination (~30%) (Harrison *et al.* 1990). Repeated exposure to highly irradiated cercariae was also protective against *S. mansoni* (Stek *et al.* 1981b) and could be boosted by repeated exposures (Kariuki *et al.* 2004). Significant partial protection (~45%) was also demonstrated in chimpanzees, our closest primate relative (Eberl *et al.* 2001).

So, high levels of protection have been demonstrated in a wide variety of hosts against various schistosome species including the three major human species. Choice of optimal radiation dose and use of

multiple vaccinations are more crucial for some species than others, notably primates.

INDUCERS AND TARGETS OF IMMUNITY

Nearly all of our further understanding of the mechanisms of immunity induced by irradiated infections has come from studies of *S. mansoni* in mice, and to some extent from rats. The fact that optimal doses of radiation result in migration to and death in the lungs focused attention on the immunogenicity of the lung-stage schistosomula (LS) and led to experiments showing that irradiated LS introduced into the lungs via intravenous injection (Dean *et al.* 1981; Ford *et al.* 1984*a*), or into the skin (Coulson and Mountford, 1989) could confer immunity. Emphasis on the larval stages was further supported by the relative failure of irradiated 3- or 4-week-old worms (Dean *et al.* 1981) or unirradiated adult worm (single sex) infections to induce protection in mice (Bickle *et al.* 1979*a*).

The LS was also shown, in the majority of studies, to be the principal target of immunity in challenged animals. By recovery of parasites following mincing and incubation of tissue (Minard *et al.* 1978*b*; Stek *et al.* 1981*a*) by quantitative histology (Mastin *et al.* 1983; Von Lichtenberg *et al.* 1985) and most accurately by autoradiographic tracking in compressed organs (Dean *et al.* 1984; Wilson *et al.* 1986; Dean and Mangold, 1992) it was shown that migration of percutaneously applied challenge larvae to the lungs is delayed in vaccinated compared with naïve mice but otherwise comparable. Subsequently, a greater proportion of schistosomula are retained in the lungs of vaccinated mice and these gradually disappear between 2–5+ weeks post-infection (Wilson *et al.* 1986; Dean and Mangold, 1992). LS introduced by i.v. injection into the lungs of vaccinated rats (Ford *et al.* 1984*b*; McLaren *et al.* 1985) and mice (Mangold *et al.* 1986) were also highly susceptible to attrition although, in some experiments in mice, somewhat higher levels of resistance followed percutaneous challenge (Smythies *et al.* 1996; Coulson and Wilson, 1997) which the authors suggested indicates that the skin stage may help to initiate a recall response prior to lung attrition.

Histological studies of the response elicited by percutaneously applied challenge infections in vaccinated mice showed pronounced inflammatory foci around schistosomula in the skin and subsequently in the lungs (Von Lichtenberg *et al.* 1985), for example, by day 8 post-challenge there were >10-fold more lung foci in vaccinated compared with control mice. The cellular infiltration, comprising predominantly mononuclear cells, starts as soon as the larvae reach the lungs and peaks around 8 days after infection (Smythies *et al.* 1996). Cells accumulate within the blood vessel and between the vascular endothelium and the alveolar epithelium resulting, it is suggested,

in the intravascular migration of the larvae being impeded. Certainly, with time, an increasing proportion of larvae occur within alveoli where they induce even more pronounced foci (Crabtree and Wilson, 1986). Although Von Lichtenberg *et al.* (1985) described damaged LS in vaccinated mice, Crabtree and Wilson (1986) found no evidence of parasite damage and this led to the notion that LS may not be killed within the lung but rather diverted from their intravascular migration into the alveoli and subsequently lost via the trachea and eventually the GI tract. This was supported by later studies showing that challenge LS recovered from lungs of vaccinated mice over the extended period of attrition showed normal viability when injected into naïve mice (Coulson and Wilson, 1988). Furthermore, radio-isotope labelled, challenge organisms in compressed lungs always appeared as discrete autoradiographic foci of equal intensity, unlike foci caused when radio-isotope labelled, heat-killed LS were injected into the lungs which became smaller and fainter with time (Dean and Mangold, 1992). The lack of evidence of *in vivo* death of LS is consistent with the demonstration that they are also unsusceptible to immune damage *in vitro* by either antibody- or cell-mediated mechanisms (Moser *et al.* 1980; Sher *et al.* 1982*b*; Bickle and Ford, 1982; Ahmed *et al.* 1997).

MECHANISMS OF IMMUNITY

Initial investigations into the mechanisms of vaccine-induced resistance in rodents implicated the immune response since B cell-depleted mice and athymic mice and rats failed to develop resistance (Sher *et al.* 1982*a*; Ford *et al.* 1987*a*). Both antibody and CD4⁺ T-cell-mediated, IFN- γ -dependent effector mechanisms have been demonstrated, depending on the host species and the number of vaccinations.

In mice, larvae from a percutaneously applied, optimally irradiated infection undergo a protracted migration in the skin (Mangold and Dean, 1984), specifically in the skin draining lymph nodes (SLN), and release greater amounts of parasite antigen than unirradiated larvae (Mountford *et al.* 1988). This results in marked increases in cell number (Constant *et al.* 1990) and prolonged *in vitro* antigen-specific CD4⁺ cell proliferation which is followed some time later by similar events in the lungs and draining mediastinal lymph nodes as larvae migrate there (James *et al.* 1981; Lewis and Wilson, 1982; Pemberton *et al.* 1991). Initial percutaneous exposure to either normal or irradiated cercariae induces both Th1 (IFN- γ and IL-2) and Th2 (IL-5 and IL-4) cytokine responses in the SLN (Caulada-Benedetti *et al.* 1991) but these are more protracted with the irradiated infection and Th1 cytokines responses tend to increase preferentially at later time points (Pemberton *et al.* 1991). Th1-polarized

cytokine production is more prominent in the spleen (Caulada-Benedetti *et al.* 1991; Pearce *et al.* 1991; Pemberton *et al.* 1991) and the lungs (Smythies *et al.* 1992*b*). Based on an extensive series of studies (reviewed by Coulson, 1997) Wilson and colleagues determined that the cells primed in the SLN enter the systemic circulation and are recruited as effector/memory cells to the lung airways and draining mediastinal lymph nodes, this recruitment coinciding with cell accumulation around irradiated larvae reaching the lungs (Mastin *et al.* 1985). This cell recruitment to the lung is dependent on the vaccinating larvae reaching the lungs and is essential for optimal immunity since naïve mice parabiosed to vaccinated partners show transfer of antigen-specific cells to the spleen but not to the lung, and manifest lower resistance (mean 35% lower) than their vaccinated partners (Coulson and Wilson, 1997). In contrast to the latter, the parabiosed mice are also unable to resist an intravenous challenge with LS which is consistent with the concept that optimally irradiated larvae result in 'arming' of the lung with cells able to respond rapidly to migrating LS (Wilson and Coulson, 1989). These experiments and others (Mountford *et al.* 1992) highlight the fact that the high levels of immunity which can be induced in mice by a single exposure to attenuated larvae crucially depend on a proportion of the larvae surviving to reach and die in the lungs.

Cell-mediated immunity (CMI)

A role for Th1-mediated immunity in mice exposed once to irradiated cercariae was first indicated by the failure to induce resistance in CMI-deficient P strain mice (James and Sher, 1983) and later confirmed by the observation that significantly lower resistance occurred if CD4+ cells were depleted at the time of challenge (Kelly and Colley, 1988; Vignali *et al.* 1989*a*). After challenge of vaccinated mice, bronchoalveolar lavage (BAL) cells increase markedly in the lung and IFN- γ production from CD4+ cells dominates, peaking at day 14 as foci develop around challenge parasites (Smythies *et al.* 1992*b*). Marked increases in IFN- γ , IL-12 p40, TNF- α , and iNOS mRNA expression occur (Wynn *et al.* 1994, 1995) and high levels of iNOS mRNA occur specifically in the parasite-associated inflammatory foci (Wynn *et al.* 1994). BAL cells also show highly elevated nitric oxide (NO) production to a challenge of LS administered *i.v.* (Coulson *et al.* 1998). However, experiments designed to determine the contribution of IFN- γ and NO to protection in singly vaccinated mice have reached somewhat varied conclusions. Depletion of IFN- γ during challenge with neutralizing monoclonal antibody has been reported to dramatically (Smythies *et al.* 1992*a*) or modestly (Wynn *et al.* 1994; Jankovic *et al.* 1999) reduce protection. Similarly IFN- γ Receptor KO mice show

marked reduction ($\sim 55\%$) in protection (Wilson *et al.* 1996) whereas IFN- γ KO mice show a modest reduction (25%) (Jankovic *et al.* 1999). Use of iNOS KO mice or inhibitors of NO production have led to the conclusion that NO plays a modest role (reducing protection by $\sim 30\%$) (James *et al.* 1998; Wynn *et al.* 1994) or no significant role (Coulson *et al.* 1998) in protection. Overall, these studies support a significant if not dominant role for CMI in singly vaccinated mice but Hewitson *et al.* (2007) have recently questioned this since mice lacking CD154, which are deficient in both Th1 and IgG responses, fail to develop protection when the Th1 component is reconstituted by administration of IL-12.

Antibody-mediated immunity

Antibody contributes to immunity following single vaccination in mice since protection is significantly ($\sim 30\text{--}50\%$) lower in B-cell-deficient (μ MT) compared with WT mice (Anderson *et al.* 1999; Jankovic *et al.* 1999) and transfer of serum from singly vaccinated WT donors restores μ MT immunity to that in WT mice (Jankovic *et al.* 1999). Following repeated exposure of mice CMI becomes less important and antibody more so. CD4+ cell depletion has less effect on protection after double vaccination (Vignali *et al.* 1989*a*) and, following multiple (5 \times) vaccination, depletion has no effect which, together with much enhanced antibody levels, indicates that any CMI requirement is fully replaced by antibody-mediated protection (Kelly and Colley, 1988). This is consistent with the demonstration by Caulada-Benedetti *et al.* (1991) that following repeated (3 \times) exposure Th2 cytokines are upregulated at the expense of Th1 cytokines. Repeat vaccination also leads to somewhat enhanced protection (Minard *et al.* 1978*a*; Richter *et al.* 1993*a*; Anderson *et al.* 1999; Jankovic *et al.* 1999) and to boosting of antibody titres (Delgado and McLaren, 1990; Caulada-Benedetti *et al.* 1991; Anderson *et al.* 1999; Jankovic *et al.* 1999). This boosting of the antibody response explains the increased protection since both occur in IFN- γ KO but not in B-cell-deficient mice (Anderson *et al.* 1999; Jankovic *et al.* 1999).

The ability of attenuated larval infections to induce protective antibody was demonstrated directly by passive transfer of protection with serum from rabbits vaccinated with 20 krad-irradiated cercariae (Ford *et al.* 1984*b*; Bickle *et al.* 1985). Homologous passive transfer of protection using sera from singly or multiply vaccinated rats indicated that antibody is the principal mediator of protection in rats (Ford *et al.* 1984*b*, 1987*b*). Such serum transfer is equally or more effective at day 5, when the larvae are in the lungs, than at the time of challenge, but less effective when the larvae reach the liver (Ford *et al.* 1984*b*; Bickle *et al.* 1985; Mangold and Dean, 1992). This demonstrates that irradiated vaccine-induced

antibody is effective against LS which were first shown to be a target of immunity in passive transfer experiments using serum from rats infected with unattenuated larvae (Mangold and Knopf, 1981). Using a different strain of rat McLaren and Smithers (1985) found that passive transfer of vaccinated rat serum was effective at day 5 post-challenge but not at day 0 and was also effective against a challenge of LS delivered into the lung via tail vein injection if given on the same day but not 4+ days later. This demonstration of the unique susceptibility of the lung stage is consistent with the observed lung-stage attrition of the challenge infection in vaccinated rats (Ford *et al.* 1984*b*). The protection was shown to be mediated by IgG2a (Ford *et al.* 1987*b*), the major isotype in rats (Bazin *et al.* 1974) and by IgG in rabbit serum (Mangold and Dean, 1992).

Homologous passive transfer in mice was first shown by Mangold and Dean (1986) (50–85% transfer of the resistance of the donors). Successful transfer depended on the donor mice receiving repeat (2 or more) vaccinations and, for optimal protection, for the serum to be given at the lung stage (6–10 days post-challenge) rather than at the time of challenge. Similar results were obtained by Richter *et al.* (1993*a*) and for *S. japonicum* by Moloney and Webbe (1990). However, McLaren and Smithers (1988) who, in contrast to other workers (Mastin *et al.* 1983; Von Lichtenberg *et al.* 1985; Dean *et al.* 1984; Wilson *et al.* 1986; Dean and Mangold, 1992) had described challenge parasite attrition in the skin of vaccinated mice (Kamiya *et al.* 1987) reported markedly higher protection when serum was transferred at the skin rather than at the lung stage. Various studies have shown that IgG1, IgG2a and IgG2b are boosted following multiple vaccination of mice although IgG1 is the most prominent isotype (Delgado and McLaren, 1990; Caulada-Benedetti *et al.* 1991; Wynn *et al.* 1996). By passive transfer, IgG (Mangold and Dean, 1986) and specifically IgG1 (Delgado and McLaren, 1990) have been identified as protective isotypes but the non-IgG serum fraction has also been implicated (Jwo and LoVerde, 1989).

In primates, repeated exposures are needed to induce modest to high levels of protection and result in boosting of antigen-specific IgG, levels of which correlate with protection (Harrison *et al.* 1990; Soisson *et al.* 1993; Yole *et al.* 1996). This indicates that antibody plays a major role in irradiated vaccine immunity in primates.

Regarding the mechanism of antibody-mediated attrition, mice vaccinated with repeat (2 or 3) exposures and in which antibody is believed to play a key role, show essentially similar mononuclear cell-rich foci around challenge LS in the lung (Vignali *et al.* 1988*a*; Kassim *et al.* 1992) as have been described for singly vaccinated mice (Crabtree and

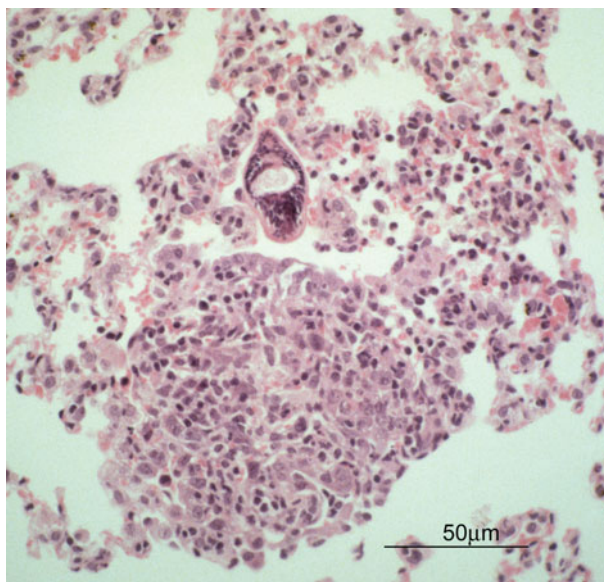


Fig. 1. Antibody-mediated mononuclear cell infiltration around a lung-stage schistosomulum induced by homologous passive transfer of vaccinated rat serum.

Wilson, 1986). Again the larvae appear undamaged and a proportion of them occur within alveoli, in which case they induce marked inflammatory responses (Kassim *et al.* 1992). McLaren and Smithers (1988) reported that transfer of immune serum at the skin stage elicited inflammatory reactions of mononuclear cells and eosinophils around schistosomula in the skin which, nevertheless, appeared morphologically similar to LS and so even in this model of apparent skin phase attrition, lung-form larvae appear to be the target. In rats, where there is consensus that challenge attrition occurs in the lungs of vaccinated animals (Ford *et al.* 1984*b*; McLaren *et al.* 1985) passive transfer of immune serum to naïve rats 5 days post-infection, when the challenge parasites are in the lungs, elicits an enhanced and accelerated inflammatory mononuclear cellular infiltration around the larvae compared with normal serum (Vignali *et al.* 1989*b*) as shown in Fig. 1. Because in mice there is no evidence of parasite damage or of intimate cellular attachment, cellular accumulation interfering with parasite migration (Crabtree and Wilson, 1986) is a plausible cause of parasite loss. How IgG is able to mediate such inflammatory foci is unknown. Protection occurs in Fc γ R KO mice (Jankovic *et al.* 1999) and so Fc-receptor-mediated activation of cells for ADCC is not required. In rats, complement depletion during passive transfer at the lung stage significantly reduces protection (Vignali *et al.* 1988*b*) and so generation of the chemoattractants C3a and C5a by antibody/antigen fixation of complement may play a role in focus formation, at least in the rat. Other possible roles of antibody in interfering with parasite migration/viability by binding to surface receptors or signalling molecules remain to be assessed.

THE ANTIGENS INVOLVED

Although not all studies agree, the weight of evidence from mice and rats is that the lung schistosomula alone can induce high levels of protection and that they are the only necessary targets eliciting cell- or antibody-mediated reactions during lung transit. It follows that the protective antigens are those responsible for the induction of lung foci such as shown in Fig. 1. Antigens responsible for T cell-mediated focus formation would be soluble or secreted antigens available to antigen presenting cells. Antibody could bind to such secreted antigens but could also bind to exposed epitopes of integral membrane antigens. Although the LS shows minimal surface antigenicity as judged by immunofluorescence (Pearce *et al.* 1986) sera from vaccinated animals can mediate transient cell adherence (Bickle and Ford, 1982; Lawson *et al.* 1993) indicating that antibody does have access to exposed larval antigens.

It follows that investigation of the vaccine potential of the secreted and membrane antigens of LS is a rational approach and soluble extract of *in vitro*-derived LS is indeed able to induce significant protection in mice (Mountford *et al.* 1996). To characterize the LS antigens Harrop *et al.* (1999) used antisera against products released by such larvae in culture to screen Western blots of cercarial, LS and adult antigens. No LS-specific antigens were detected and when the sera were used to screen cDNA libraries most clones corresponded to previously described immunodominant antigens whose representation in the LS culture supernatant may have been due to release from damaged larvae. Cercariae, *in vitro*-derived schistosomula and adult worms are much more ready sources of antigen for study than *in vivo*-derived LS, but whether these stages share the protective LS antigens involved in irradiated vaccine immunity or whether these are transiently expressed during larval development *in vivo* is uncertain. However, strong protection (~90%) can be induced in mice with repeated (5 ×) exposure to very highly irradiated (e.g. 100 krad) cercariae which die in the skin (Hsu *et al.* 1981) and comparable levels of protection to those demonstrated with LS (Mountford *et al.* 1996) can be induced with cercarial, *in vitro*-derived schistosomula or adult antigens given with BCG intradermally (James *et al.* 1985). So the early larval and adult stages clearly manifest antigens which can induce protection. Recently, newer technologies for characterizing membrane and secreted antigens have been applied to analysis of schistosome adult worm proteins. Rigorous proteomic analysis has led to identification and characterization of a large number of adult tegumental (Braschi *et al.* 2006) and surface-associated (Braschi and Wilson, 2006) proteins. Cloning of genes with signal sequences has allowed identification of adult proteins targeted for secretion or surface expression (Smyth

et al. 2003). Application of such methods has recently led to identification of tegumental proteins able to induce protection in mice e.g. the tetraspanins, TSP-1 and TSP-2 (Tran *et al.* 2006) and Sm29 (Cardoso *et al.* 2008). Their involvement in irradiated vaccine immunity and exposure on the surface of living worms remains to be established although Sm29 has been shown to be expressed by the LS tegument (Cardoso *et al.* 2008).

Various approaches have been applied to identify antigens specifically recognized by vaccinated animals e.g. characterizing larval surface antigens recognized by vaccine serum (Simpson *et al.* 1983); generating monoclonal antibodies against larval surface antigens using vaccinated donors (Bickle *et al.* 1986; Dalton *et al.* 1987); testing known recombinant-derived antigens for reactivity with antibody or cells from vaccinated mice (Richter and Harn, 1993; Richter *et al.* 1993b); identification of recombinant-derived antigens uniquely or preferentially recognized by irradiated vaccine serum (compared with infection serum) (Soisson *et al.* 1992; Francis and Bickle, 1992). Some of these antigens were included in the independent vaccine testing (Bergquist and Colley, 1998) but others remain to be characterized and tested. Amongst these are larval surface antigens uniquely recognized by vaccine serum but not by infection serum, e.g. 15 kDa (Simpson *et al.* 1983) and 16 kDa (Bickle *et al.* 1986) antigens and which appear to have transient expression in the larval stages (Bickle and Oldridge, 1999). Definition of such larval surface antigens will likely need application of those techniques so successfully applied to the adult tegument. It should be noted that it is not yet clear whether any of the above antigens or the many other schistosome antigens identified by other means, play a significant role in irradiated vaccine-induced immunity and specifically in mediating lung focus formation.

PROSPECTS FOR A DEFINED VACCINE

The evidence from the irradiated vaccine models suggests that provided the immunogenic moieties can be produced, protective immune responses may not be particularly challenging to induce. Thus, although WT mice, which make a balanced Th1/Th2 response, develop optimal immunity, high partial protection occurs in cytokine KO mice whether Th1- or Th2-polarized responses are induced (Hoffmann *et al.* 1999).

To simulate the antibody-mediated protection induced by irradiated vaccines IgG antibodies will be needed rather than IgE antibodies which are implicated in naturally-acquired resistance in humans (Hagan *et al.* 1998). Longevity of sufficiently high antibody titre will be a requirement. Based on irradiated vaccine studies in various host species, immunizing regimens which are believed to induce

antibody-mediated immunity have been shown to lead to long-lived protection (Dean 1983; Hsu *et al.* 1981; Bickle *et al.* 1979*d*; Ford *et al.* 1984*a*) although some decline in protection with time has been shown in baboons (Harrison *et al.* 1990).

A vaccine aimed at reproducing the CMI mechanisms which help mediate protection in singly vaccinated mice faces a number of challenges. It would need to induce the lung sensitization required for optimal immunity in this model and this is likely to require targeting vaccine delivery to the lung as proposed, for example, for TB vaccines (Kallenius *et al.* 2007). It would need to induce Th1 responses but avoid over-induction of regulatory cytokines e.g. IL-10, since this can prevent manifestation of resistance (Oswald *et al.* 1998). Challenge exposure downregulates the Th1 response in mice (Pemberton and Wilson, 1995) and in the likely event of a vaccine inducing only partial protection the ensuing egg production is likely to profoundly alter the response from a Th-1 to a Th2-dominated response, as occurs when schistosome eggs are injected into vaccinated mice (Pearce *et al.* 1991). Furthermore, in endemic areas, vaccinees are likely to have an underlying Th2 biased response due to other worm infections or to previously treated schistosome infections that could interfere with the generation of Th1 responses. Regarding the influence of current/previous infection on vaccine efficacy it is encouraging that studies in baboons have shown that multiple irradiated vaccine regimens, likely to induce antibody-mediated protection, are equally effective in naïve and in infected or infected and previously treated animals (Kariuki *et al.* 2006).

Given the availability of a new crop of promising protective antigens and the enthusiastic application of new technologies for indentifying membrane and secreted antigens a schistosome vaccine modelled on the irradiated vaccine seems increasingly technically feasible. However, as Loukas *et al.* (2007) urged, development of this promise into a human vaccine will require the wheel to turn and significant investment to return to schistosome vaccine development.

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