Strategies for a schistosome vaccine: can we manipulate the immune response effectively?

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1. What is the rationale for developing a schistosome vaccine?

Vaccines arguably represent the most effective means to control diseases caused by infectious agents. However, in the case of schistosomiasis we might ask ‘Why attempt to develop a vaccine when there is an effective chemotherapeutic agent in the form of Praziquantel?’ A compelling reason is the cryptic nature of the schistosome infection and associated disease processes. In contrast to e.g., cutaneous leishmaniasis or malaria, the immediate manifestations of schistosomiasis for most people are negligible or indeterminate; the infected individual is therefore unaware of the accumulating worm burden. These facts, coupled with the normally slow progression of the pathological state, mean that 5 to 10 years may elapse after first exposure before the hepatosplenic condition becomes apparent. At this late stage, chemotherapy can do little to reverse the pathology.

One attribute of chemotherapy is that it reduces the intensity of infection and overall prevalence to the point where, hopefully, few individuals progress to the advanced disease state. However, to sustain these effects it must be applied systematically on a large scale, irrespective of infection status, and perhaps for an indefinite period (cf. fascioliasis in the UK; [55]). The risk associated with mass treatment is the development of drug resistance by the parasite and, in this context, a low efficacy of Praziquantel treatment has already been reported in Egypt and Senegal. Indeed, experiences with malaria and gastrointestinal nematodes suggest that the selection of drug-resistant schistosome strains is almost inevitable. For this reason, we should use the window provided by Praziquantel to intensify our search for a vaccine.

There is a further potent argument for a vaccine which, up to now, has received little consideration. The immunodepressive nature of a schistosome infection is evident from both human and animal studies; if the mechanisms involved are nonspecific then there are obvious implications for the host’s ability to resist concurrent infections, as already revealed by altered murine responses to vaccinia virus [1]. We consider that these three aspects of parasite-host interactions provide the strongest rationale for the development of a schistosome vaccine.

2. Can responses to a schistosome infection provide the basis for a vaccine?

If we accept that a vaccine would be a desirable tool, then what strategy should we follow in order to obtain one? It must be remembered that most currently available vaccines for use in humans have been devised empirically and are directed against diseases with an acute rather than chronic time course. If a solid immunity develops as a consequence of acute infection, the likelihood of achieving an effective vaccine is high. With chronic infections, the vaccinologist starts at a disadvantage in that the existence of a protective immune response is by no means clear or guaranteed. We have recently addressed the issue of why, in spite of 30 years’ research, there is no vaccine against schistosomiasis [54]. In this article, we focus on schistosomiasis mansoni and vaccine approaches to achieve a reduced worm burden rather than anti-fecundity or anti-pathology effects.

An obvious starting point for a vaccine would be to replicate the protective immunity which a schistosome infection elicited in the human host, but a major problem has been to demonstrate the existence of such protection. The most persuasive evidence has come from studies of reinfection after curative chemotherapy, which reveal lower rates in older age groups, independent of water contact [22]. However, recent interpretations of these data suggest that the sharp decline in reinfection rate coincides with puberty [23]. This might have an immunological basis, but equally could depend on hormonal or other changes. Furthermore, the age group most in need of protection (2- to 15-year-olds) shows little evidence of acquired immunity. Even if it could be demonstrated that the older age groups become immune, the most likely protective mechanism advanced to date is mediated by IgE [19]. It is difficult to envisage how such a mechanism could be replicated using current vaccine technology without a risk of dangerous side effects such as increased allergy. Taking all of the above into account, it seems very unlikely that in the short term a viable strategy for vaccine development will emerge from studies of infected humans.

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Schistosoma mansoni will infect a wide range of laboratory hosts and so there is no shortage of animal models in which to study mechanisms of acquired immunity which might form the basis of a vaccine. Indeed, early studies led to the concept of concomitant immunity in which a primary worm burden remained but the host was ostensibly resistant to reinfection. Unfortunately, in the mouse the phenomenon has shown a strong association with egg-induced porta-caval anastomoses, which prevent establishment of challenge worms in the portal system (reviewed in [53]). Thus, even if protective immune mechanisms are in operation, it is impossible to pinpoint them against the background of altered migration kinetics. What is needed is an infection model in which acquired immunity can be disassociated from haemodynamic changes. In this context, the rhesus monkey, in which concomitant immunity was originally demonstrated, would repay further investigation to determine whether there is a true immunological basis for the phenomenon. The olive baboon may also merit attention because there is some evidence that it does not develop porta-caval anastomoses during chronic infection [51]. An advantage of both primates is their size relative to laboratory rodents, which means that they can harbour a more realistic schistosome worm burden; a disadvantage is the paucity of reagents to facilitate analysis of host immunological responses. There is presently insufficient information from these primate hosts on which to base a vaccine strategy.

3. Does the laboratory rat provide a novel route to a vaccine?

The laboratory rat represents a special case of a semi-permissive host in which self-cure occurs approximately 28 days after a primary infection; it is thereafter strongly immune to challenge. The phenomenon appears to be species-specific because the black rat (R. rattus) does not self-cure in the same way [28]. Although nutritional and immunological explanations have been proposed for the phenomenon, a clear association between an increase in total IgE and a decrease in worm burden over the period of self-cure favours the latter [14, 48]. Using an in vitro mast cell degranulation assay, we have shown that schistosome-specific IgE levels also rise over the period of worm elimination in the liver [14]. Prior to self-cure, there is mast cell recruitment to the hepatic parenchyma, a process which does not occur in the permissive murine host [36]. Furthermore, degranulation of mast cells, evidenced by release of rat mast cell protease II into the bloodstream, coincides with elimination of the primary worm burden in the hepatic portal distributaries. The close approximation of degranulated mast cells and dying worms could imply that mast cell mediators are, directly or indirectly, the agents of worm death. Conversely, we cannot rule out the possibility that some other mechanism causes worm damage, thus releasing antigens; mast cell degranulation would then merely be its sequela.

If we accept that self-cure is the result of an immediate hypersensitivity reaction, it must be inferred that the rat can generate IgE antibodies to normal adult worm products. Two dominant allergens of 67 and 36–38 kDa have been detected on Western blots of adult worm antigens probed with day 35 infection serum [14]. Periodate treatment of the antigen preparations strongly inhibits mast cell degranulation in vitro, suggesting that the relevant antigen epitopes are glycans. This is perhaps not surprising, since degranulation requires the cross-linking of Fcε receptors which could be accomplished by repeated glycan epitopes.

It has been proposed, on the basis of passive and adoptive transfer experiments, that the acquired immunity subsequent to self-cure involves IgE associated with effector leukocytes in antibody-dependent cellular cytotoxicity mechanisms directed against incoming larvae [5]. Why the rat should mount such a Th2-biased response to schistosome infection, in comparison to the mouse or other hosts, remains to be explained, but must reside in the interaction between the products of developing larvae and cells of the antigen-presenting pathways. Taken together, the above facts provide strong circumstantial evidence for the operation of IgE-mediated mechanisms in elimination of both primary and challenge worm burdens in the laboratory rat. If this is the way that the rat achieves mastery over a schistosome infection, it is difficult to envisage how the mechanisms could be replicated in a vaccine for use in humans, because of the requirement for antigen-specific IgE. In the case of self-cure, the need for mast cell recruitment to the liver and the involvement of glycan epitopes are an added complication.

4. The radiation-attenuated (RA) schistosome vaccine: an alternative approach

If a vaccine strategy based on responses elicited by schistosome infection looks doubtful, does the alternative approach of generating a response quantitatively or qualitatively different from that seen after infection offer a greater potential? This idea is not as implausible as it might seem. For example, vaccination of mice with recombinant glutathione S-transferase in adjuvant elicits a far higher antibody titre to the protein than does a schistosome infection [32]. The RA vaccine does not appear to suffer from the limitations of strategies based on human or laboratory animal infections, and we have suggested that it too elicits novel responses [54]. Both rodents and primates exposed to cercaria larvae, optimally attenuated with gamma radiation, show a highly significant reduction in challenge worm burden compared to controls (reviewed in [7]). In the C57BL/6 strain mouse given a single exposure to the RA vaccine, this amounts to 60 to 70% protection, but multiple vaccinations do not result in sterile immunity. The work of the York group over the last 15 years has focused on understanding the mechanisms operating in both the induction and effector phases of immunity, with the ultimate aim of replicating them in a recombinant antigen vaccine. In this model of protection, challenge worm elimination takes place predominantly in the lungs [7]. Administration of anti-CD4 monoclonal antibodies around the time of challenge to mice given a
single vaccination has shown that Th cells are essential to protection, implying a cell-mediated effector mechanism involving delayed-type hypersensitivity (DTH; [52]). Furthermore, analysis of the lungs has revealed a focal inflammation, largely mononuclear cell in composition, around challenge larvae lodged in the vasculature [13].

4.1. Th subset differentiation

The development of the Th1/Th2 paradigm for Th cell differentiation has had a profound influence on our attempts to understand the RA vaccine model. Originally defined in a panel of long-term murine T helper cell clones on the basis of their cytokine secretion profiles [37], these two phenotypes have since been confirmed in mouse immune responses to infections [38] and amongst T cells from human patients [47]. This similarity implies that technologies developed in murine models would potentially be transferable to humans. Broadly speaking, the two major patterns of cytokine synthesis initially recognised appear to correlate with the induction of cell-mediated (Th1) or humoral (Th2) immunity, thus providing a possible explanation for the separate and often reciprocal differentiation, together with subsequent Th0 (unrestricted cytokine profile; [21]) and Th3/Tr1 (downregulating IL-10 and transforming growth factor-beta (TGF-β) production; [6, 25]) extensions, provides the framework within which we can evaluate the mechanisms operating in vaccine-induced immunity. Moreover it offers, by manipulation of the cytokine milieu, the means to bias an immune response in a desired direction.

4.2. Priming for a Th1 response

In the context of the Th1/Th2 paradigm, one of the first questions we addressed was the nature of the primary response elicited by the RA vaccine. The attenuated larvae undergo a truncated migration in the mammalian host, with a proportion remaining in the skin, and approximately 10% entering and persisting in the skin-draining lymph nodes; the remainder exit the skin via blood vessels but travel no further than the lungs. Antigen stimulation of lymphocytes from skin-draining lymph nodes at day 5 postexposure results in a mixed (Th0) cytokine profile. Subsequently, the response polarises in a Th1 direction with interferon gamma (IFN-γ) as the dominant cytokine [43]. The way in which the attenuated larvae stimulate this Th1-biased response is the subject of ongoing investigations. The induction process must be initiated by parasitc constituents (presently uncharacterised) released during penetration of the skin or in the days thereafter. These, in turn, have to interact with the accessory cells to influence the expression of costimulators (e.g., B7.1, B7.2), and/or release of cytokines which contribute to the milieu in which antigen presentation to T cells takes place. A recent immunocytochemical analysis of leucocyte-parasite interactions in the skin has revealed the potential for antigen processing by MHC II+ CD11c+ dendritic cells or macrophages over a protracted period in this site [46]. Such cells may also translocate to the draining nodes for antigen presentation. In addition, larval persistence in the nodes may be akin to the effect of an adjuvant in prolonging the period of immune priming.

With regard to the cytokines which potentiate a Th1 response, IL-12 and IL-18 are the most likely candidates. We have not been able to determine a requirement for IL-18 due to lack of reagents. However, the involvement of IL-12 has been examined using mice in which the gene for the p40 chain of this cytokine has been disrupted [2]. At day 5 after vaccination, antigen-specific production of IFN-γ was dramatically lower in these mice than in wild-type counterparts, whilst an early shift to a Th2 profile was illustrated by increased levels of IL-4, IL-5, and IL-10 in lymph node cultures. Thus, IL-12 is required to prime the Th1 response in this model. However, the site of IL-12 production and the cells of origin remain to be determined.

The attenuated larvae which migrate to the lungs also play an important role in the primary immune response through their actions in stimulating lymphocyte recruitment to that organ. From approximately 1 to 3 weeks after vaccination a population of schistosome-specific, DTH-mediating CD4+ T cells can be detected in the circulation [45], coincident with the sequestration of attenuated larvae in the lungs. The outcome is the recruitment to the pulmonary parenchyma and airways of a population of CD44hi CD45RBhi memory/effector cells with Th1 characteristics [9, 49]; experiments involving parabiotic mice have revealed that these resident cells are important agents initiating an effector response against challenge larvae [10].

4.3. The cell-mediated pulmonary effector mechanism

The operation of a T-cell-mediated DTH response against a large multicellular parasite is perhaps rather surprising given that this mechanism is usually deployed against intracellular pathogens such as Mycobacterium and Leishmania. In the case of these organisms, the core of the mechanism is the presentation of antigen to specific effector T cells which release IFN-γ, and this in turn activates infected macrophages to kill the pathogen. In the RA vaccine model, we have shown that antibodies to IFN-γ, administered to vaccinated mice from four days after challenge, reduced protection by 90%, indicating a key role for this cytokine in the effector response [50]. For the intracellular pathogens, one function of IFN-γ is the upregulation of inducible nitric oxide synthase (iNOS) to generate nitric oxide (NO), a crucial cytotoxic agent [24]. It is thus reasonable to ask whether NO is involved in the elimination of challenge schistosomes despite their size and extracellular location. Indeed, NO has been proposed as a killing agent on the basis of its schistosomucidal properties in vitro [29]. We have therefore investigated the potential in vivo role of NO in the elimination of challenge parasites from the lungs of vaccinated mice.

The upregulation of iNOS mRNA and the production of NO by airway leucocyte cultures suggest that the basic conditions for NO to act as a larvicidal agent in the lungs are fulfilled [11]. However, neither administration of L-NMMA, an inhibitor of iNOS activity, nor vaccination of mice with a disrupted iNOS gene resulted in abrogation of protection. Whilst NO from activated macrophages will indeed kill newly transformed schistosomula in vitro, the NO scavenging effects of haemoglobin, achieved by the
addition of a small quantity of erythrocytes to the larvicidal assays, completely abolishes its lethal effects. We interpret this to mean that once larvae enter the bloodstream they will be protected from the cytotoxic action of NO. In addition, since the lung stage larvae have a virtually anaerobic metabolism, unlike newly transformed skin schistosomula, they do not present oxidative enzyme targets for the inhibitory actions of NO. Our data thus provide little evidence to implicate NO as a major component of the pulmonary effector response.

Earlier observations on the lack of damage to challenge larvae trapped in pulmonary foci [13], and the viability of such larvae revealed by transfer experiments [8], led us to suggest that the effector mechanism does not operate by cytotoxic killing but rather by simple physical blocking of parasite migration. Support for this hypothesis was provided by experiments on mice; in which IFN-γ had been neutralised [50] or the gene for the IFN-γ receptor was disrupted (IFN-γR−/− mice; [57]). In both cases, low levels of protection were associated with the presence of larger and more diffuse foci in the lungs, presumably providing less impediment to larval progress through the pulmonary vasculature. This suggested to us that adhesive interactions between cells might regulate the compactness and effectiveness of pulmonary foci, particularly as IFN-γ is a known stimulator of adhesion molecule expression [30]. Whilst we have been able to demonstrate reduced ICAM-1 expression on CD4+ T cells from the lungs of IFN-γR−/− mice, we found that protection was not reduced in mice lacking intercellular adhesion molecule 1 (ICAM-1) [56]. If focus integrity is central to the operation of the effector response, then we have yet to identify the crucial interactions involved.

Another putative function of IFN-γ is to stimulate the synthesis by macrophages of tumour necrosis factor (TNF), a cytokine with pleiotropic actions. Evidence that this occurs in the RA vaccine model is provided by the higher levels of TNF-α mRNA detected in the lungs of C57BL/6 mice after challenge, compared to IFN-γR−/− mice [57]. We have therefore determined whether TNF has a role in the effector response by vaccination and challenge of mice with a disruption in the gene for the p55 receptor of TNF (TNFR−/− mice). This produced the striking result of a complete loss of protection phenotype (unpublished data), and we are currently trying to establish how TNF operates. The one major discrepancy between TNFR−/− mice and their wild-type counterparts is a low antibody titre in the former, due to defective germinal centre formation in lymphoid tissue [33], and could imply that, in spite of an absolute requirement for CD4+ T cells in the effector response, there is an ancillary role for antibody. However, vaccination of MT mice which lack functional B cells has revealed that the majority exhibit levels of protection identical to their C57BL/6 wild-type counterparts [Anderson et al. Immunology 96 (1999): 22–28], and confirms that cell-mediated mechanisms are the dominant force after a single vaccination.

4.4. Antibody-mediated protection

As a corollary to the above, serum from such C57BL/6 mice confers only low levels of protection on naïve recipients. However, multiple exposures to attenuated cercariae generate a small increase both in antibody titre and the efficacy of passive transfer [34]. This suggests that the dominant Th1 response is limiting the expansion of a Th2-mediated antibody response by the cross-regulatory actions of IFN-γ. We have tested this hypothesis by exposing IFN-γR−/− mice to multiple vaccinations, the outcome being an increase in the level of protection obtained with each successive vaccination (unpublished data). There is a concomitant rise in total IgE levels and schistosome-specific IgG1 titres, both indicative of a dominant Th2 response in these animals. Furthermore, serum from multiply-vaccinated IFN-γR−/− donors will confer 60% protection on naïve C57BL/6 recipients. To date, we have not found any evidence in either the serum donors or recipients for challenge parasite elimination in the lungs; this suggests that antibody-mediated mechanisms operate at a different site to cell-mediated mechanisms, quite possibly the skin. (One caveat for any effector mechanism acting shortly after penetration is that the parasite may have moved on, geographically or developmentally, before an anamnestic response gets underway.)

In conclusion, we interpret the results from the RA vaccine experiments to mean that a vaccine strategy with the relevant antigen(s), designed to promote either a cell-mediated or a humoral response, should elicit a high level of protection.

5. Does parasite-induced immunoregulation impact on vaccine strategies?

The effect of a schistosome infection on the mammalian host must be a consideration in any strategy for vaccine development, because of the likelihood that in an endemic area all but the youngest children will have already been exposed. It is evident that schistosome parasites, and not just their eggs, provoke cellular and humoral responses. Indeed, after surgical transfer of adult male worms to the portal system of naïve mice, we have used the antibody responses to identify a group of secreted antigens, which proved to originate in the gut and tegument [15]. Given the continuous accumulation of worms in untreated children, it is reasonable to assume that the immune response to these antigens is insufficient to provide protection. It is also evident that, by comparison with attenuated larvae, invading normal larvae do not appear to be very immunogenic [43]. It must be remembered that the Th1/Th2 cytokine profiles were delineated using T-cell clones, and in most instances in vivo responses to infection are seldom polarised to the same extent. We infer that this is the case with normal schistosome parasites which provoke an ineffective Th0 response, prior to egg deposition. Furthermore, there is evidence from earlier studies that schistosome infections in mice profoundly depress splenocyte proliferation in response to mitogens [41] and irrelevant antigens [3]. This effect is most marked after egg deposition but occurs in mice with chronic unsexual infections [17]. Egg deposition is also followed by declining Th1 and increasing Th2 cytokine production by both
parasite antigen- and mitogen-stimulated splenocyte cultures [26]; a similar switch in host responses to an unrelated antigen has been reported [31]. It is not possible to follow the progression of human responses to schistosome infection in the same way as for mice, because of the ethical requirement to treat patients. However, it is clear that individuals in the early, acute phase of schistosomiasis have vigorous T-cell proliferative responses compared to those with a chronic infection, as reported by e.g., Yazdanbakhsh (personal communication) for S. haematobium. In this study a difference in the pattern of cytokine secretion by antigen-stimulated peripheral blood mononuclear cells was also noted, with more IFN-γ in the former and more IL-5 in the latter group of patients. The inference must be that progression from the acute to the chronic phase of the disease in humans is associated with a reduction in the blastogenic potential of T lymphocytes, accompanied by a shift towards the Th2 pole.

There are several ways in which a schistosome infection could cause immunodepression or subvert the T helper response. One might be by stimulating production of downregulatory cytokines such as IL-10 or transforming growth factor-β. We have recently demonstrated both specific and nonspecific immunodepression in S. haematobium-infected mice after the onset of egg deposition, associated with enhanced IL-10 production by splenocyte cultures [32]. Furthermore, addition of neutralising anti-IL-10 antibodies to peripheral blood mononuclear cell cultures from patients with chronic intestinal schistosomiasis mansoni dramatically increased the proliferative responses to adult worm and egg antigens. However, the same procedure had no effect on antigen-specific proliferation in cultures from patients at the acute stage of infection [44]. Clearly IL-10, via its action on antigen-presenting cells, could represent an agent for the generalised inhibition of Th1 responses. In addition, activation-induced apoptosis would provide a mechanism for the elimination of schistosome-reactive T cells [20].

These data suggest that schistosomes, via their products, actively manipulate the host’s immune status; if correct, this supposition has profound implications for vaccination of humans. The occurrence of immunodepression would provide a powerful argument for developing a vaccine for administration to young children so as to minimise the impact of a schistosome infection on their overall immunological status. For already infected individuals, it would be necessary not only to eliminate the worm burden but also to allow for recovery of immune responsiveness before vaccination could take place.

6. The ‘Happy Valley’ hypothesis

Our observations on vaccine-induced protection and speculations on worm-induced immunodepression have led us to propose the ‘Happy Valley’ hypothesis of immunity to schistosomes. It provides a paradigm to explain why the development of a vaccine has proved so problematic. We can envisage the host’s immune response as a linear axis (figure 1), with a Th1 phenotype at one pole and a Th2 at the other. The parasite appears most comfortable in the Th0 zone represented by the ‘valley’ floor. The special attribute of the RA vaccine is to shift the response away from the intermediate Th0 position leading to protection, represented by the walls of the ‘valley’. The largest shift in a Th1 direction occurs in the C57BL/6 mouse strain, the highest responder to the vaccine identified to date. IL-10 production, concomitant with IFN-γ after challenge, appears to be a major influence retarding movement towards the Th1 pole [42]. (Other cases have been described in which IL-10, though posited as a Th2 cytokine, is produced in parallel with IFN-γ, e.g., [58].) However, the position of the response along the axis can be manipulated, for example using recombinant cytokines. There are two reports where the immunity induced in C57BL/6 mice has been significantly amplified by coadministration of the RA vaccine with recombinant IL-12 [2, 59]. Whilst we are currently investigating the effects of vaccination in mice with a disrupted IL-10 gene, the ultimate Th1-biased mouse would be incapable of responding to IL-4, IL-13 and IL-10.

By comparison, it is more straightforward to drive the response towards the Th2 pole, by removing the inhibitory effects of just one cytokine, IFN-γ. However, multiple vaccinations are needed to achieve high levels of protection, resulting in higher antibody titres than in similarly treated C57BL/6 mice. There appears to be a ceiling to antibody-mediated protection (although we have not attempted any cytokine interventions to boost Th2 responses in IFN-γR-/- mice) which may result from an inability to maintain high titres for prolonged periods, a suggestion for which we have direct evidence from exposure of primates to the RA vaccine [60].

If our interpretation is apposite, how do we formulate recombinant antigens for administration to an immunologically intact animal to obtain an extreme bias in the Th1 or Th2 arm of the response? (It seems unlikely that both options would be possible simultaneously.) We have made a start by demonstrating that a crude antigen preparation from lung worms is ineffective alone but elicits significant protection when coadministered with recombinant IL-12 [40]. The IL-12 treatment, whilst boosting IFN-γ production in antigen-stimulated lymph node cultures, depressed IL-10 and virtually abolished IL-4. An indirect approach would be to administer with the antigen, ligands which instructed accessory cells to produce the requisite stimulatory cytokines in vivo. In this context, the characterisation of pathogen-derived molecules which bias the T helper responses is still in its early stages.

7. Identification of protective antigens

Given that the RA vaccine will, in appropriate circumstances, elicit either Th1- or Th2-mediated responses, do we have any information on the antigens relevant to protection in the model? The most intensively scrutinised group of antigens is the panel of six vaccine candidates selected by WHO for detailed evaluation [4]. It is not clear whether any of them are key mediators of the protection induced by the RA vaccine, although one (a fragment of
myosin) was identified using serum from mice exposed to irradiated cercariae [16]. However, we can make some inferences about the antigens involved in the cell-mediated (Th1) protective response elicited in the lungs by the RA vaccine. Since the effector mechanism in this situation is T helper cell-dependent, the antigens must be released from the viable lung-stage parasite for processing by accessory cells before presentation of peptide fragments bound to MHC class II [39]. The incipient parasite gut and the syncytial tegument provide potential sources of such antigens. The direct contact between the putative accessory cells and the parasite surface in lymph nodes during priming suggests that tegumental antigens may be crucial immunostimulators [46]. Moreover, biosynthetic labelling of larvae grown to the lung stage in vitro has revealed de novo synthesis and secretion of several proteins [27].

We have no similar pointers to the source of antigens for antibody-mediated protection elicited by the RA vaccine. Indeed, we have not identified a distinct site of action or defined a mechanism. However, if we make an assumption that the skin-stage parasite is the target, the antigens must either be accessible on its surface or released from it. If the former, then the mechanism most probably involves opsonisation, complement fixation, or leucocyte adherence, leading to cytotoxic killing of the parasite. If the latter, then immune complex formation may be the agent stimulating inflammation which, as with the lung stage, blocks migration. There are several cellular structures in the cercaria/schistosomulum which could serve as a source of protective antigens. The pre-and postacetabular gland secretions are used by the parasite for penetration of the stratum corneum to gain access to the epidermis; the gland cells disappear within 24 to 48 h. The cercarial tegument membrane is shed immediately after penetration and replaced by the complex multilaminate surface [35] which, by analogy with what occurs in the adult worm, is thereafter in a state of continuous turnover. Finally, secretions from the head gland are used by the larva to penetrate the basement membrane of the epidermis to gain access to the dermis, and again to penetrate the blood vessel wall [12].

These inferences about mechanisms and antigens raise a general point. The larval structures involved were all

Figure 1. The ‘Happy Valley’ hypothesis. The schistosome larva/worm appears to provoke a relatively ineffective Th response with a mixed (Th0) cytokine profile (subsequently modified by egg deposition). A vaccination protocol with relevant antigens, e.g., the RA vaccine, which achieves a significant deflection of the Th response in either a Th1 or Th2 direction, will produce a measure of protection. The greater the bias towards one or the other pole, the higher the level of that protection. This presumably reflects either the increasing frequency of specific responder T cells or the concentration of effector antibodies. The IFN-γR−/− mouse represents the extreme Th2 pole of antibody-mediated protection. By analogy, an IL-10−/−/IL-4R−/− mouse should represent the extreme Th1 pole. At present, the nearest approach to that pole has been achieved with C57BL/6 mice receiving the RA vaccine plus recombinant IL-12.

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characterised by electron microscopy in the 1970s and 80s and have since been neglected. The analogous situation in apicomplexan protozoa led to the development of antibody reagents which were used both to identify structural components of e.g., the merozoite surface or rhotries, and to obtain recombinant proteins by cDNA library screening. At present, with the exception of cercarial elastase released from the acutabular glands, there are few if any authenticated markers for schistosome glands or epithelia.

A major limitation of cDNA library screening using infection serum, or even antibodies which will confer protection, seems to be that a very small subset of antigens (e.g., paramyosin, hsp70) is identified. These antigens probably represent the most abundant mRNA/protein species in the parasite and, for a variety of reasons, they are unlikely to be involved in protection elicited by the RA vaccine [18, 54]. A screening approach which identifies scarce secreted membrane antigens in cDNA libraries needs to be developed. Only then are we likely to obtain sufficient quantities of the relevant recombinants for evaluation in protection experiments.

8. Conclusions

It is clear that the RA vaccine will, under appropriate conditions, elicit partially protective Th1 or Th2 responses. In theory, if such responses were directed against different parasite stages at different sites, they might act additively or even synergistically, to provide a very high level of protection. The demonstration of this effect in rodent and/or primate models would strengthen the case for this conclusion. However, the responses might prove mutually exclusive, in which case it would be necessary to opt for the one which gave the maximum level of protection. A second important step forward would be the identification and cloning of the antigens which mediate the respective Th1 and Th2 mechanisms induced by the RA vaccine. The task would then be to find ways of formulating the relevant antigens to invoke the desired responses, perhaps by means of two distinct and sequential vaccination procedures.

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