

# Schistosomiasis Vaccine Development: Approaches and Prospects

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*Mounting evidence for acquired immunity to schistosomiasis in humans supports the case for immunological intervention. On the other hand, rapid reinfection poses a threat to younger age groups due to the slow maturation of natural resistance. However, rational approaches, based on advances in immunology and molecular biology, have substantially increased the odds of producing an effective vaccine. Since the parasite cannot replicate in the human host and serious morbidity generally occurs only after a relatively long period of heavy worm burden, complete protection against infection is not essential. The chances of success would increase if more than one of the various host/parasite interphases were targeted, for example reducing morbidity through decreased worm loads as well as through suppression of egg production.*

*Several promising schistosome antigens have now reached an advanced phase of development and are currently undergoing independent confirmatory testing according to a standardized protocol. A few molecules are being contemplated for scaled-up production but, so far, only one has reached the stage of industrial manufacture and safety testing. Since schistosomiasis cannot realistically be controlled by a single approach, vaccination is envisaged to be implemented in conjunction with other means of control, notably chemotherapy.*

Key words: antigen - cytokine - schistosomiasis - *Schistosoma* - immunity - protection - resistance - vaccine

The ubiquity of reinfection after chemotherapy for schistosomiasis, particularly in young inhabitants of endemic areas, is a compelling reason for developing a long-term measure to complement drug treatment. Propelled by the accumulation of positive data on human acquired immunity from many geographical areas endemic for *Schistosoma mansoni* and/or *S. haematobium* (Hagan et al. 1991, Hagan & Abath 1992, Dessein et al. 1992, Butterworth et al. 1992), and by encouraging protection experiments in animal models with improved antigens, vaccine development must now be accepted as a realistic approach.

Although gaps persist in our model of the development of pathology and resistance in schistosomiasis, a consolidated picture of the disease as the result of an interplay between opposing immunological mechanisms is emerging. Caught between the need to prevent further invasion of parasites and the necessity to modulate the granulomas which form around eggs trapped in host tissues, the immune system displays a bewildering activity of various effector cells and antibody isotypes. The main obstacle resides in the fact that parasites, in contrast to the great majority of other infectious agents, commonly strike a balance with their hosts, and one which does not exclude reinfection. In the case of schistosomiasis,

however, several factors confer a comparative advantage, justifying the continued support of schistosomiasis vaccine development: (a) diverse protective mechanisms have been demonstrated in experimental animals; (b) naturally acquired partial immunity is the most plausible explanation for the observed reduced intensity of infection in adolescent humans and older age groups compared to children; (c) complete immunity is not required since the parasite does not replicate in the human host and the aim is confined to prevention of morbidity; (d) safe and effective drugs are available.

Native antigens seem to provide superior protection but for reasons of safety and production parasite vaccines should be made from recombinant ones. Based on the assumption that candidate antigens are sufficiently cross-reactive to be effective against several species, it is prudent to work initially with *S. mansoni* whose life-cycle can easily be maintained in the laboratory. However, as it is only logical to expect that homologous antigens would produce improved protection, antigens of other schistosome species will eventually have to be developed. In fact, an impressive number of *S. japonicum* antigens has already been cloned and expressed (Waine et al. 1993). The possibility of finding new superior antigens cannot be ruled out but it makes sense to now move

on to human trials with those molecules which are ready, not least because this would help elucidate how resistance against schistosomiasis develops in man, something which is still largely unknown.

This paper emphasizes the advantages of a concerted approach to vaccine development geared at simultaneous interference with several stages of the parasite life-cycle.

**ANTIGEN COMPOSITION**

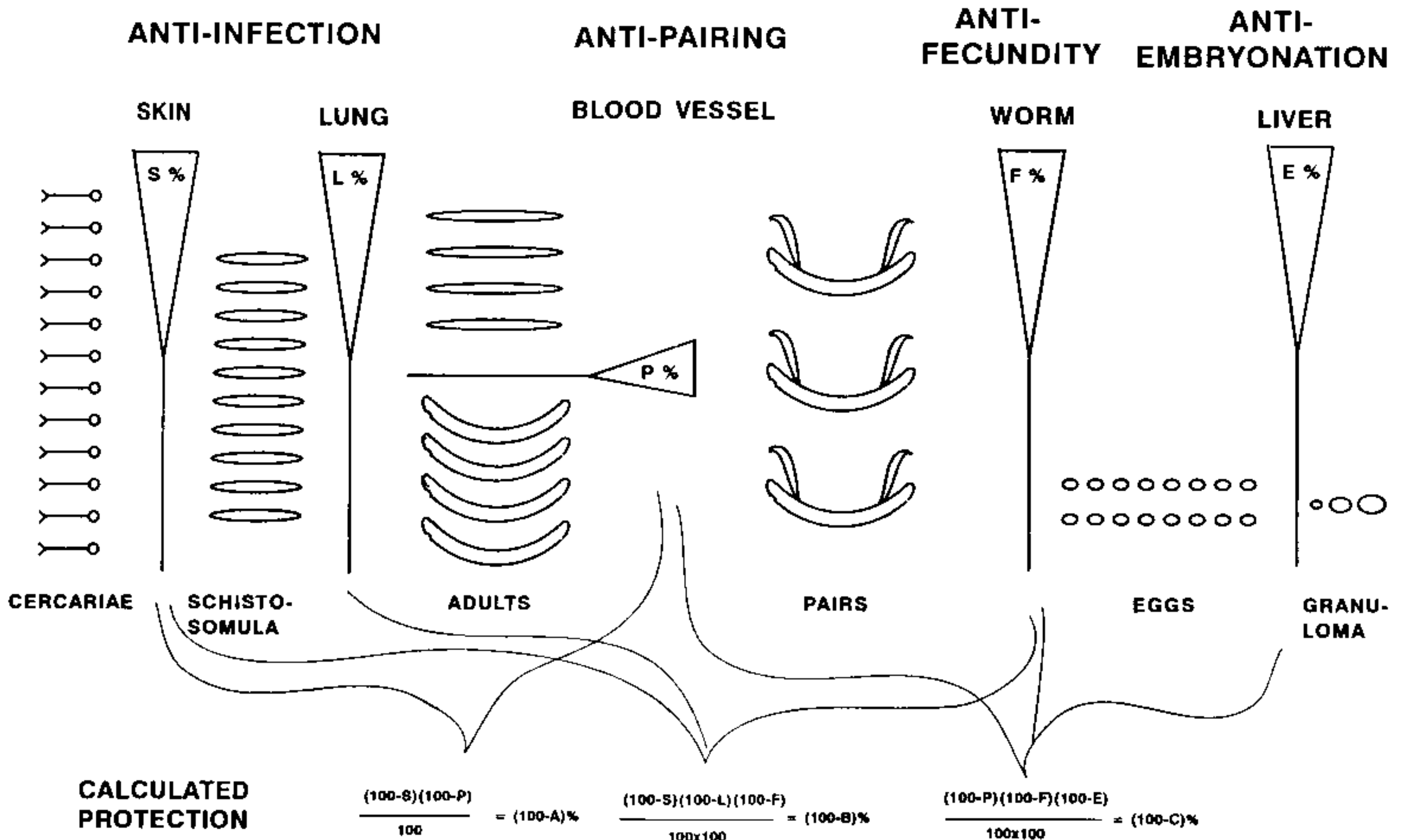
The biochemical nature of epitopes does not have implications only for immunogenicity. For example, carbohydrate antigens may augment the risk of inadvertently activating the granulomatous reaction since they often cross-react with egg antigens (Simpson 1989). The contrast when comparing the relatively small granulomas of chronic schistosomiasis with the large, florid ones associated with the early stage of infection (Domingo & Warren 1968, von Lichtenberg 1987) and the fact that anti-carbohydrate antibodies eventually become down-regulated (Omer-Ali et al. 1989), accentuate the dualistic operation of the immune system. Antibodies which block the protective responses in *S. mansoni* (Butterworth et al. 1992) and *S. haematobium* (Hagan et al. 1991, Hagan et Abath 1992), probably constitute a related issue.

**VACCINE TARGETS**

The total number of cloned schistosome antigens, expressed in various microorganisms such

as viruses, yeast and bacteria, including BCG, may soon reach 100 but only a few of them show genuine promise. The schistosomulum surface is the preferential antigen source but all stages of the parasite life-cycle in the vertebrate host may be targeted. Attempts to combine full-length protein antigens from the same stage have not been encouraging but the use of antigens from diverse stages might improve the possibilities for synergistic action by involving separate defence mechanisms. Table gives the current status of different approaches to obstruct the progress of the parasite, and Figure illustrates how consolidated levels of protection with several disparate antigens can be gauged. The arbitrarily chosen examples show that three antigens, each producing 25% protection, would together only allow about 40% of cercariae to develop to adult worms, whilst two antigens would require 40% protection each to surpass that. A brief discussion of potential components of a combination vaccine follows below.

*Skin penetration* - Six cercarial antigens have been described. One is a 41 kDa antigen proposed for diagnosis of acute schistosomiasis (Hayunga et al. 1986) and the other five are proteases, measuring 25 kDa (Landsperger et al. 1982), 28 kDa (Marikovsky et al. 1990), 30 kDa (McKerrow et al. 1985), 41 kDa and 47 kDa (Chavez-Olortegui et al. 1992), respectively. These enzymes display diverse substrate specificities but have a strong immunogenicity in common, suggesting a possible role in vaccine development. Surprisingly, none



Mechanisms of protection.

TABLE

## Defence Mechanisms and Intervention Targets

Parasite activity	Target	Identified	Cloned	Tested
Cercarial invasion	Antigens	Some	One	None
Larval growth	Antigens	Many	Many	Yes
Worm pairing	Pheromone	None	None	None
	Receptor	None	None	None
Egg production	Antigens	One	Yes	Yes
Egg maturation	Miracidial secretion	None	None	None

of them have been seriously studied as possible candidate vaccine antigens.

*Larval development* - The schistosomulum undergoes a phase of rapid growth from the time immediately after cercarial penetration until early maturity about three weeks later. The literature contains numerous references to surface antigens which range from 8 to >200 kDa and are often shared with other stages, most commonly with the adult worm and the egg (Simpson 1989, 1990). In general, the levels of protection displayed in permissive animals after immunization are not impressive and since it is well known that almost complete protection can be induced by cercariae attenuated by sub-lethal doses of radiation, the search for superior antigens continues. It should be noted, however, that this effect may not be due to the exposure of particular antigens *per se* but rather reflect improved antigen presentation due to a prolonged passage through lymph nodes and lungs. On the other hand, the significance of epitope configuration is emphasized by the demonstration of improved protection induced by some "new" antigens, including a 62 kDa myosin-like protein implicated in the irradiated cercariae model (Soisson et al. 1992) and Sm14 (Moser et al. 1991, Tendler et al. 1994) which have both shown protection in the 50-70% range in mice. Other effective, well-explored antigens associated with the schistosomulum include paramyosin (Pearce et al. 1988), Sm23 (Reynolds et al. 1992, Köster et al. 1993), triose-phosphatase isomerase (TPI) (Shoemaker et al. 1992, Reynolds et al. 1994) and, in particular, glutathione S-transferase (GST) (Balloul et al. 1987, Capron et al. 1992).

*Pairing* - *In vitro* experiments have shown that the two sexes of schistosome do not attract each other before the fourth week of development, whilst older specimens orient themselves to the opposite

sex to form egg-producing pairs (Eveland & Haseeb 1989). More recent research suggests that females exhibit a receptor capable of responding to a lipid-containing compound released from the male from the fourth week after infection (Haseeb & Eveland 1991). Identification of the receptor or the pheromone is a long-term endeavor but progress in this area could lead to a way of effectively blocking egg production, the major problem in schistosomiasis.

*Fecundity* - Even after pairing has taken place, the production and release of eggs from the female schistosome may be successfully interfered with. Almost two decades ago, an anti-fecundity effect mediated by immune responses was reported in experimental studies on *S. haematobium* infection in baboons (Webbe et al. 1976) and later confirmed in cattle infected by *S. bovis* (Bushara et al. 1980). A fresh surge of interest in exploiting this potentially useful mechanism has brought these results to the forefront in vaccine discussions (Xu et al. 1991, Boulanger et al. 1991, Agnew et al. 1993). Interestingly, immunization of mice with *S. mansoni* GST indicates that this antigen not only protects against infection in the traditional sense but also reduces egg production by up to 85% (Xu et al. 1991, Boulanger et al. 1991).

*Granuloma formation* - The early recognition of the phenomenon of granulomatous regulation (Domingo & Warren, 1968) opened up the prospects of anti-pathology vaccination, leading to the hypothesis of anti-embryonation (Garcia et al. 1982). This approach attempts to reduce immune-mediated pathology by halting egg maturation by vaccination with viable immature eggs, thereby curtailing the release of immunogenic compounds from the miracidium inside. Garcia et al. (1989) were able to show that *S. japonicum* eggs mature in lower numbers in chronically egg-sensitized

mice and that sera from humans with chronic schistosomiasis are capable of reducing granuloma development. A beneficial effect on granuloma size after injection of irradiated schistosomula in baboons has been reported by Damian et al. (1984) and a similar but not statistically significant effect was noted after experimental vaccination with GST (Boulanger et al. 1991). However, as egg antigens are not involved in this case, the effect is probably not due to anti-embryonation.

In an attempt to modulate the cell-mediated response to schistosomal antigens at the clonal level,  $T_H1$  cell clones directed against egg antigens have been raised and their targets identified (Chikunguwo et al. 1992, Stadecker, 1992, Flores-Villanueva et al. 1994). Further work along these lines may make it possible to induce anergy by blocking the events leading to the granulomatous reaction.

#### CYTOKINE REGULATION

The recently acquired ability to study the triggering of immune responses at the molecular level *in vivo* has revolutionized the study of host defence mechanisms. Resistance to infection in general is controlled by two pivotal sub-populations of T helper cells,  $T_H1$  and  $T_H2$ , the former predominantly activating cell-mediated responses through the production of interferon-gamma (IFN- $\gamma$ ), interleukin two (IL-2) and IL-12, whilst the latter is associated with IL-4 and IL-5 and the stimulation of antibodies including the immediate hypersensitivity and eosinophilia characteristic of helminth infection. The unravelling of these mechanisms in schistosomiasis opens the road to modulation of the immune response. For example, the injection of irradiated *S. mansoni* cercariae into mice results in a  $T_H1$  response correlated to resistance, whilst normal cercariae induces a  $T_H2$  response which is essentially without protection (Pearce et al. 1991, Grzych et al. 1991). The protective responses have been demonstrated to correlate with the activation of macrophage effector cells by IFN- $\gamma$  (James, 1992). Interestingly, the transfer of eggs to vaccinated mice incurs a switch from the  $T_H1$ -dominated response to one mainly consisting of  $T_H2$  cells which seem to permit both infection and further egg deposition (Grzych et al. 1991).

IL-4, IL-10 and transformation growth factor-beta (TGF- $\beta$ ) are potent down-regulating cytokines capable of inhibiting schistosomal destruction as shown by the fact that even suboptimal combinations of any two of these cytokines synergistically suppress parasite killing by IFN- $\gamma$ -activated macrophages (Oswald et al. 1992). For example, the supernatant from a line of spleen cells from a mouse infected with *S. mansoni* was reported to

inhibit the growth of a culture of  $T_H1$  cells, which effect could be blocked by the addition of antibodies against IL-10 (Sher et al. 1991). In mice receiving macrophages isolated from egg granulomas IL-4 and IL-10 increased while IL-2 decreased to undetectable levels (Flores-Villanueva et al. 1994). It is even possible that schistosomula use  $T_H2$  activation as a strategy to evade macrophage-mediated killing. On the other hand, anti-parasite IgG, IgE and IgA antibodies correlate with resistance to reinfection in drug-cured patients and eosinophils have been shown to mediate IgE and IgG<sub>2</sub>a antibody-dependent cell-mediated cytotoxicity against schistosomula *in vitro* (Capron et al. 1992, Dunne et al. 1992).

IL-12 displays a strong T cell effect (Perussia et al. 1992) which could possibly be utilized for  $T_H1$  stimulation in future vaccines but since this cytokine also seems to down-regulate IgE responses (Kiniwa et al. 1992) there is again a balance to be struck. The growing awareness of the need to consider all aspects of the immune system, encourages the use of antigens expressing B cell epitopes as well as T cell epitopes. Most antigens proposed for vaccine studies contain both and some vaccine candidates consisting of synthetic peptides mimicking such epitopes have been produced and tested (Auriault et al. 1990, Reynolds et al. 1992, 1994).

The granulomatous response to schistosome eggs appears to have a strong  $T_H2$  component, and its development is counteracted by treatment with anti IL-4 antibody (Chensue et al. 1992). The fibroblast-stimulating factor-1 (FsF-1), a fibrosis-stimulating cytokine produced by CD4+ cells residing in granulomas (Prakash & Wyler 1992) is another possible target for immunologic intervention. Finally, tumour necrosis factor (TNF)- $\alpha$  may play an important role in schistosome associated pathology since infection of the severe combined immunodeficiency (SCID) mouse followed by addition of the recombinant cytokine stimulates egg production and restores granuloma formation (Amiri et al. 1992).

The role of the  $T_H1/T_H2$  cell balance in humans with schistosomiasis remains unclear. On the one hand, a reverse balance of IFN- $\gamma$ /IL-4 persisting at least three months after cure, disturbed expressions of the ratio of CD3+ to CD8+ cells and a reversible decrease of IL-1 were reported in Brazilians infected with *S. mansoni* (Zwingenberger et al. 1989a,b, 1990). On the other, high levels of specific IgE antibodies correlate with post-treatment resistance in cohorts of Africans infected with *S. mansoni* or *S. haematobium* (Hagan et al. 1991, Dunne et al. 1992).

#### CONCLUSIONS

More emphasis on research into cercarial penetration of the host skin is warranted as an im-

proved early impediment would reduce the number of developing schistosomes, thus facilitating implementation of preventive measures geared at the later stages. Combined antigens from different parasite stages should result in superior resistance and the less the relation between the responses elicited the better the chance of inducing mutually bolstering mechanisms. Rational antigen combination would permit also the utilization of antigens of modest protective value. It is, in this connection, interesting to note the natural combination of two, perhaps even three, separate effector mechanisms in the GST molecule.

The risk of vaccine interference with the beneficial regulation of granuloma formation makes it necessary to avoid activating epitopes involved in this pathway. Even if it would be useful to reinforce natural down-regulation of the granuloma, this process might produce potentially harmful results if carried too far. For example, the toxic effects of leaking egg products could damage surrounding hepatocytes in the absence of any sequestering reactions. Stimulation of T cell anergy is an interesting approach but research is still at the basic stage.

Antibody-mediated blocking of pairing of the two sexes is perhaps the most elegant way of avoiding pathological reactions due to schistosomiasis. Once the components of the chemical signaling system have been identified and characterized, generation of neutralizing antibodies against the pheromone, which probably avoids immune recognition due to its small size, would be straightforward. Alternatively, antibody-blocking of the receptor may be attempted. It is unfortunate that this potentially important area has so far attracted comparatively little attention and is consequently still far from applied research.

There is obviously dependable support for the involvement of both cell-mediated and humoral mechanisms in schistosome infection but the complex regulation of specific immune responses clouds the picture. The coexistence of activated macrophages, different kinds of T-cells and antibodies, both effective and blocking ones, does not permit a simple solution. The enigma of how resistance is generated and maintained can only be penetrated further by initiating human Phase I/II vaccine trials. Safety is assured by the availability of efficacious drugs with few side effects. The degree of protection where a beneficial effect would be clearly observed can be estimated to be above 50% and is probably below 80%. Since intervention at this level would not arrest transmission, natural infection may be counted on to boost the artificially induced resistance. However, it might also be necessary to address the possibility of a slow build-up of the quantity of parasites to a signi-

ficant level in subjects who are in regular contact with water containing infected snails. In addition, females tend to increase their individual egg production when their number in the host is reduced (Hayunga et al. 1985). For these reasons, chemotherapy will almost certainly have to be continued after vaccination in many areas, albeit with much longer intervals between treatments than called for in current schedules.

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