Schistosome vaccines

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Summary. Schistosomiasis control currently relies primarily on chemotherapy which is both expensive and temporary. There is an urgent need for an effective vaccine. Studies in animal models and man have demonstrated the existence of protective immunity. Antibody-dependent cell-mediated cytotoxicity mechanisms involving eosinophils and macrophages have been implemented in destruction of the parasites. Antigens expressed on the surface of the schistosomulum are among the targets of protective immune responses. Vaccines comprising recombinant antigens are now being tested in vivo for their capacity to evoke protective responses. Live oral vaccines based on attenuated *Salmonella* expressing schistosomular surface antigens are being developed.
Key words. Schistosomiasis; immunity; antigens; vaccines.

Introduction

Schistosomiasis remains a major public health problem in over 70 countries with an estimated 200 million people infected and a further 500 million at risk. The disease is caused by digenetic trematodes of the genus *Schistosoma.* Three principle species are recognised: *S. mansoni* (Africa and South America), *S. haematobium* (Africa and the Middle East), and *S. japonicum* (Asia). Much current schistosomiasis research is concerned with the development of experimental vaccines. In this review attention is drawn to developments in areas of schistosome immunology and molecular biology that provide grounds for optimism about the eventual production of an effective vaccine against the disease.

Control methods

Current strategies for the control of schistosomiasis are based primarily on chemotherapy and, to a lesser extent, on the use of molluscicides and on water management designed to modify adversely the environment of the snail intermediate hosts. In all cases, such measures are temporary and expensive. For example, in areas of high transmission, reinfection can occur very rapidly and consequently an effective chemotherapy programme may require repeated and indefinite surveillance and retreatment. The cost of initial diagnosis, drug purchase and delivery, and surveillance may easily overrun the entire health care budget of some of the poorer countries where schistosomiasis is endemic. A further consideration is the possible development of drug resistance by the parasite. Although resistance has not been reported in the case of praziquantel, the current drug of choice, it has developed against oxamniquine, which is still widely used to treat infections with *S. mansoni.*

In contrast, the major advantage of a vaccine is that once immunity has developed, it could be long-lasting, and it seems reasonable to expect that a single immunization would be sufficient to protect an individual living in an endemic area. The development of a live vaccine, perhaps based on transformed attenuated *Salmonella* given as a single oral dose, could provide the ideal delivery system. However, before this stage is reached there are several very important questions that must be answered. First, how do adult schistosomes survive for such long periods in an apparently immunocompetent host? Second, can protective immunity be demonstrated in either experimental animals or man? Third, at what stage of the parasite's life cycle and by what mechanism is protective immunity effected? Fourth, what are the antigens against which putative protective responses are directed? Some answers are available.

Parasite immune evasion mechanisms

Early experiments carried out by Smithers and Terry[49, 50] demonstrated the ability of an established primary infection to evoke an immune response capable of destroying a secondary challenge. These experiments identified the young schistosomulum larvae as a possible target for protective responses, but they also raised questions about the mechanism by which adult worms can survive in the face of a potentially lethal immune attack. The passive absorption of host molecules which mask the parasite's surface antigens was one of the first mechanisms suggested to explain immune evasion. However, this idea must be modified to take account of the observation that schistosomula maintained in vitro in defined media show an age-dependent increase in resistance to antibody-dependent eosinophil-mediated killing[19]. Analysis of purified tegumental membranes, together with comparison of the surface labelling patterns obtained at different times after transformation of cercariae to schistosomula, suggests that there is a progressive loss of antigens from the outer of the two bilipid layers that comprise the surface tegumental membrane[45, 51, 53]. It would appear, therefore, that schistosomes have an intrinsic capacity to evade their host's immune defenses, although a secondary role of absorbed host molecules is not ruled out.

Immune responses in animals

Despite the existence of parasite immune evasion mechanisms, both rodents and primates have been shown to develop a strong immunity to challenge infections. Extracts and homogenates of parasites have been used to immunize animals, with varying degrees of success; however, the most effective protection is obtained by immunization with irradiated cercariae[4, 5]. An irradiation dose of about 20 Krads is used to attenuate the parasites, which must survive for at least 7–14 days to evoke an effective immune response. However, the precise site of attrition is debatable, with the skin, lungs and liver being identified as primary sites in various model systems[44, 60]. Direct evidence that the young schistosomulum is a target for protective immune responses comes from experiments performed with monoclonal antibodies. For example, Smith and colleagues[46], demonstrated the capacity for an IgM monoclonal antibody, with specificity for schistosomulum surface carbohydrate epitopes, to protect mice from infection in passive transfer experiments. This report was soon followed by others involving other antibody isotypes with specificities for peptide as well as carbohydrate antigens[6, 20, 25, 28, 29, 35, 61].

Human immune responses

Evidence for the existence of immunity against *S. mansoni* in man has been obtained by Butterworth and his colleagues[11, 12], who examined the rate of reinfection of children following chemotherapy under conditions where the level of exposure was monitored. Two extreme groups were identified. First, those presenting with high levels of reinfection and therefore identified as 'susceptible', and second, those showing low levels of reinfection in spite of high exposure and therefore considered 'resistant'. The mean pretreatment intensities of the two groups were similar, but there was a marked difference in age, indicating that the observed resistance was an age-dependent event, and independent of levels of exposure. A similar conclusion was drawn by Hagan and Wilkins following their studies on *S. haematobium*[28, 29, 59].

In initial attempts to correlate specific immunological responses with development of resistance against *S. haematobium*, a possible role for eosinophils was suggested[28] (although no such correlation was found in the *S. mansoni* studies[12]). These cells, together with macrophages, neutrophils and platelets, had previously been shown to be capable of destroying schistosomula by antibody-dependent cell cytotoxicity mechanisms (ADCC)[7, 15]. As far as human studies are concerned most attention has focused on eosinophils and macrophages, while the role of neutrophils is controversial[10]. In the case of eosinophils, killing can be mediated by both IgG and IgE isotypes although their relative effectiveness is dependent on the activation state of cells. Eosinophils from normal non-eosinophilic individuals mediate killing of schistosomula with IgG only, but cells recovered from eosinophilic individuals (> 300 cells/μl), including those infected with helminth parasites, can kill schistosomula in the presence of IgE antibodies. Activation of eosinophils is dependent upon production of a soluble protein or cytokine (eosinophil activating factor, EAF) by monocytes[57].

IgE antibodies are also responsible for mediating killing by resting macrophages and platelets[15], although the mechanisms are different. In the case of macrophages, direct contact with the target is required, but platelets mediate killing through production of soluble toxins and direct contact is not required.

The importance of individual antibody isotypes in the various effector mechanisms is further highlighted by the observation that the capacity of an IgG_{2a} rat monoclonal antibody to mediate eosinophil killing of schistosomula in vitro, can be blocked by an IgG_{2c} monoclonal antibody which shares specificity for the same 38,000 Mr glycoprotein antigen[26].

A possible role for blocking antibodies in human immunity was first suggested by Khalife and colleagues[38], who demonstrated the presence of IgM antibodies which could block the eosinophil-dependent cytotoxic effect of IgG antibodies in the same sera. These blocking antibodies were found to predominate in the serum of young children who, by comparison to older individuals, are more susceptible to reinfection after treatment. A proportion of these blocking antibodies, which also include IgG_2 and possibly IgG_4 antibodies, recognise carbohydrate epitopes shared by both schistosomula and eggs[23, 41].

These observations led Butterworth and colleagues[8, 9] to put forward the following hypothesis to explain the late development of immunity in man. During early infections of young children, the major antigenic stimulus is provided by eggs, especially by high molecular weight carbohydrates, which elicit high and persistent levels of IgM (and IgG_2) antibodies which cross-react and bind to the surface of schistosomula, thereby preventing, or blocking, binding of IgG and IgE isotypes that are capable of mediating putative protective ADCC reactions. As the child grows older, synthesis of IgM and other blocking antibodies declines, so that protective IgG (and IgE) antibody responses predominate and consequently the individual becomes resistant to challenge infection.

This hypothesis also has implications for the design of an experimental vaccine. It means that particular emphasis must be put on the administration and presentation of antigen in such a way that primarily IgG_1 (and IgE) antibody responses are evoked. However, before this problem is addressed, it will be necessary to identify and produce quantities of protective antigen(s).

Candidate vaccine antigens

Radiolabelling and immunoprecipitation experiments have identified a number of schistosomular surface anti-

gens, both carbohydrate and protein in nature, which are potential vaccine candidates. Indeed, Smith and Clegg[48] have successfully immunized mice with a 155,000 Mr carbohydrate antigen, affinity-purified from adult worm homogenate using a monoclonal antibody, while Harn and colleagues[30], using a similar approach, have identified a protective 28,000 Mr protein identified as triose-phosphate isomerase. These experiments are, however, exceptional in that sufficient quantities of the antigen could be isolated directly from the parasite. A paucity of parasite material is the major obstacle to biochemical and immunological investigations of parasites. However, as far as proteins are concerned, this situation has, to some extent, been remedied by the application of molecular biology, and a number of recombinant antigens have been isolated and are now being investigated with respect to their capacity to evoke protective immune responses. The first of these recombinant antigens is a 28,000 Mr peptide, designated p28, now identified as glutathione-S-transferase[56]. This antigen appears to be synthesized by parenchymal cells and transiently expressed on the surface of the schistosomulum[2]. Antisera raised against the recombinant antigen can mediate eosinophil killing of schistosomula in vitro, while in vivo experiments have shown that rats, hamsters and baboons immunized with the recombinant develop significant levels of protection against challenge infections[2,3]. p28 is one of a family of schistosome proteins which exhibit glutathione-S-transferase activity. A 26,000 Mr enzyme, designated Sj26, has been cloned from *S. japonicum* and also shown to protect mice from challenge infections[46].

Other cloned antigens identified as possible vaccine candidates include proteins of Mr 18,000[18]; 38,000[52]; 50,000[33]; and 86,000[17,54]. In almost every case, the title of vaccine candidate is conferred on the basis of the capacity of a specific antisera or monoclonal antibody to mediate eosinophil killing of schistosomula in vitro, or to confer protection on rodents in passive transfer experiments. The ability of the corresponding recombinant antigens to evoke protective responses in animals is now being tested.

Cell-mediated immunity

ADCC mechanisms may not be the only mechanism by which invading parasites may be destroyed. Experiments performed with a 97,000 Mr recombinant antigen, identified as paramyosin[43], have led some investigators to suggest that cell-mediated immunity involving activated macrophages may play an important protective role[36,37]. Mice immunized with recombinant *S. mansoni* paramyosin exhibit a significant level of immunity against challenge infections.

Paramyosin is considered to be an internal antigen which may be actively released from migrating parasites or from dead and dying organisms. In sensitized animals, this results in a local cellular reaction capable of destroying 'bystander' parasites. Such a mechanism can clearly contribute towards pathology, but it is questionable whether it has an important protective role in human immunity, where individuals are exposed to trickle infections.

Delivery systems

A majority of animal protection experiments have been performed using a single antigen, usually administered in multiple doses in the presence of an adjuvant. This design is appropriate for animal experiments, but human trials will require consideration of a number of other factors. First, it is unlikely that an experimental human vaccine will comprise a single antigen only. The pattern of antigen recognition by sera from infected humans is heterogeneous[12]. Not all antigens evoke an antibody response in all individuals. To assure a response, it is envisaged that an experimental vaccine will comprise a mixture of antigens. A number of factors can influence the extent and nature of an antibody response and there is some indication from mouse experiments that recognition of individual schistosome antigens is influenced by the major histocompatibility complex (MHC)[39]. The extent of MHC involvement in human schistosomiasis has not been extensively studied.

Second, if a dead vaccine is employed, it may be necessary to present the antigen(s) in the presence of an adjuvant. Only alum (aluminium hydroxide) is currently used as an adjuvant in man and, although it is known to be capable of evoking good IgE responses under certain circumstances, its efficacy is dependent on the nature of the antigen[42]. A variety of experimental adjuvants are now under investigation. These include synthetic polymers, surfactants and bacterial products such as lipopolysaccharide. It is hoped that new ethical preparations will be available soon[58].

Third, multiple inoculations of a recombinant antigen preparation, perhaps given over a period of 3–6 months, could be impractical in remote areas of the developing world. More appropriate would be a single-dose vaccine, and here considerable interest has been shown in the development of live vaccines based on attenuated strains of *Salmonella*[21]. Experiments performed in our own laboratory have demonstrated the capacity of *Salmonella* transformed with recombinant plasmids to express schistosome antigens[55]. Current work in this area is concerned with the development of vectors which allow cloned sequences to be integrated into the *Salmonella*[34], and analysis of immune mechanisms, which include cell-mediated responses, evoked by immunization of experimental animals.

Finally, it should be pointed out that there is no asexual multiplication of schistosomes in man, and that pathology is directly related to worm burden. For a vaccine to be effective in preventing disease, it may be sufficient to reduce the worm burden rather than eliminate the infec-

tion completely. Continued exposure to cercarial challenge and the presence of a small number of adult worms would act as boosters.

Vaccines based on carbohydrate epitopes

The preceding discussion has been largely concerned with protein antigens expressed on the surface of the schistosomula. However, it should be remembered that over 85% of the surface of this larva is covered with carbohydrate molecules[22]. Genetic engineering cannot yet deal with carbohydrates in the relatively easy way in which it handles proteins. However, one possible solution to the problem of the production of anti-carbohydrate responses involves the use of anti-idiotypic antibodies as the immunizing antigen. The feasibility of this approach has been demonstrated by Grzych and colleagues[16,27]. These investigators prepared anti-idiotype antibodies against a monoclonal antibody with specificity for a carbohydrate determinant of a 38,000 Mr schistosomula surface glycoprotein. Rats inoculated with these anti-idiotype antibodies exhibited a significant level of protection against challenge infection.

A second approach involves possible production of so-called 'mimitopes': synthetic peptides that mimic the conformation of a native antigen. The original description of such molecules was concerned with protein antigens, but the principle should be extendable to carbohydrate epitopes[24].

Conclusions

Progress towards development of an experimental vaccine against schistosomes has been considerable over the last 5–7 years and at least one prospective vaccine candidate has now been tested in baboons[1]. This advance owes much to work in two areas. Firstly, to the extensive field studies combined with detailed immunological investigations, such as those carried out on *S. mansoni* by Butterworth and colleagues[11–13,23,38], and *S. haematobium* by Hagan and Wilkins[28,29,59], which have demonstrated the development of acquired immunity in man. Secondly, to the application of molecular biology by a large number of laboratories which are now producing the quantities of recombinant antigens required for in vivo testing. Both build on a very secure base of animal experimentation, which provides clues as to the mechanism of protective immunity and the identity of target antigens.

We now have a theoretical, but testable, design for an experimental vaccine. This will comprise a number of different antigens which are normally expressed on the surface of the schistosomulum. These antigens will be presented as recombinant molecules expressed by transformed attenuated *Salmonella*, delivered by the oral route. They should evoke an IgG (and IgE) response, and perhaps contribute to the activation of eosinophils. Only a single vaccination will be required since boosts will be provided by natural challenge.

A major part of this discussion has been based on experiments performed on *S. mansoni*. This, in part, reflects the relative ease with which this parasite can be maintained in the laboratory, in comparison to *S. haematobium* and *S. japonicum*. However, the identification of protective antigens in *S. mansoni* should help in the identification of analogous molecules in the other species. The design of a control programme for *S. haematobium* infections will have many similarities with that used for *S. mansoni*, but in the case of *S. japonicum* it must be remembered that it is a zoonotic infection and consequently it may prove necessary to include animal reservoirs in a control programme. Finally, it should be pointed out that vaccination is not regarded as a replacement for chemotherapy, but rather it is envisaged that integrated programmes combining both activities would offer the best means of control in most circumstances.

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Malaria vaccine

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Summary. Among infectious diseases caused by protozoa, malaria is still the greatest killer of children. Mortality in adults living in endemic areas is significantly lower because they frequently acquire partial or complete immunity to the major pathogen, *Plasmodium falciparum*. This natural protection indicates that vaccination may be possible, and the first candidate antigens were cloned with the use of human immune sera as probes. Genetic and biochemical analysis of the parasite proteins revealed that they are polymorphic, and frequently gene sequences were discovered which were specific for a particular parasite isolate, which eliminated most antigens for purposes of vaccine development. The most promising candidate antigens today are the major surface proteins of sporozoites and blood stage parasites. However, the immune response against those is not sufficient for complete protection, and additional, intensive research is necessary to identify new molecules to be included in a vaccine cocktail against malaria. The current spread of the disease due to increasing drug resistance of parasites and mosquito vectors emphasizes the urgent need for a vaccine.
Key words. Malaria vaccine; recombinant DNA; surface proteins; antigen polymorphism.

Malaria as a major health problem

Among the infectious diseases caused by protozoa, malaria is still the greatest killer of children, and it is one of the most incapacitating diseases of adults living in tropical and subtropical countries. The number of cases reported to the WHO in 1984 was over 100 million worldwide, and this number is still increasing. Ten Italian tourists who returned from Africa died from malaria in June 1989, which alarmed the health authorities in Europe. Symptoms such as relapsing fever or hepatomegaly appear shortly after infection with *Plasmodia*. The parasites are naturally transmitted through the bite of an infected *Anopheles* mosquito (fig. 1) but also, like other infectious agents, malaria parasites may be spread by blood transfusions or through the exchange of needles among drug addicts. Malaria tropica caused by *Plasmodium falciparum* is the most severe form of the disease, because it may lead to intravascular hemolysis and plugging of cerebral arteries. Infection by *P. vivax, P. ovale* or *P. malariae* is less deleterious and relapsing fever is frequently the sole symptom. After a fever episode, most of the patients recover and clear the parasite from their blood stream. Various eradication strategies have had only limited success, and today, the spread of the disease is increasing.

Attempts to control insect vectors

Physical protection from insect bites through nets or repellants, together with the use of effective insecticides, have significantly lowered the transmission rate of malaria. Drainage of natural or man-made mosquito breeding-places, together with a modern hygiene system and public information programs, essentially eliminated malaria from the Northern hemisphere in the first half of this century. The presence of huge natural mosquito-breeding reservoirs such as rice fields cannot be eliminated in tropical or subtropical countries for practical and economic reasons. Insecticides and drugs have failed to control the spread of the disease, owing to the development of either drug-resistant *P. falciparum* strains or insecticide-resistant *Anopheles* vectors. For example, in Brazil a DDT (di-chloro-diphenyl-trichloro-ethane) insecticide spraying program against malaria, carried out in 1969, markedly reduced the parasite transmission rate, and the average parasitemia in the population dropped in the