Development of a Vaccine Strategy against Human and Bovine Schistosomiasis. Background and Update

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Schistosomiasis is a chronic and debilitating parasitic disease that affects over 200 million people throughout the world and causes about 500 000 deaths annually.

Two specific characteristics of schistosome infection are of primordial importance to the development of a vaccine: schistosomes do not multiply within the tissues of their definitive hosts (unlike protozoan parasites) and a partial *non-sterilizing immunity can have a marked effect on the incidence of pathology and on disease transmission. Since viable eggs are the cause of disease pathology, a reduction in worm fecundity whether or not accompanied by a reduction in parasite burden is a sufficient goal for vaccine induced immunity.*

We originally showed that IgE antibodies played in experimental models a pivotal role for the development of protective immunity. These laboratory findings have been now confirmed in human populations.

Following the molecular cloning and expression of a protein 28 kDa protein of Schistosoma mansoni *and its identification as a glutathion S-transferase, immunization experiments have been undertaken in several animal species (rats, mice, baboons). Together with a significant reduction in parasite burden, vaccination with Sm28 GST was recently shown to reduce significantly parasite fecundity and egg viability leading to a decrease in liver pathology. Whereas IgE antibodies were shown to be correlated with protection against infection, IgA antibodies have been identified as one of the factors affecting egg laying and viability. In human populations, a close association was found between IgA antibody production to Sm28 GST and the decrease of egg output.*

The use of appropriate monoclonal antibody probes has allowed the demonstration that the inhibition of parasite fecundity following immunization was related to the inhibition of enzymatic activity of the molecule.

Epitope mapping of Sm28 GST has indicated the prominent role of the N and C terminal domains. Immunization with the corresponding synthetic peptides was followed by a decrease of 70 % of parasite fecundity and egg viability.

As a preliminary step towards phase I human trials, vaccination experiments have been performed in cattle, a natural model for Schistosoma bovis. *Vaccination of calves with the* S. bovis *GST has led to a reduction of over 80 % of egg output and tissue egg count.*

Significant levels of protection were also observed in goats after immunization with the recombinant S. bovis *GST. Increasing evidence of the participation of IgA antibodies in protective immunity has prompted us toward the development of mucosal immunization. Preliminary results indicate that significant levels of protection can be achieved following oral immunization with live attenuated vectors or liposomes.*

These studies seem to represent a promising approach towards the future development of a vaccine strategy against one of the major human parasitic diseases.

Key words: schistosome - vaccine - glutathione S-transferase - IgA antibodies - parasite fecundity

Schistosomiasis, the second major parasitic disease in the world after malaria affects at least 200 millions people, 500 millions being exposed to the risk of infection and is responsible for 300 to 500 000 deaths per year.

Morbidity observed in this chronic and debilitating disease is essentially related to the remarkable female worm fecundity, hundreds of eggs being laid every day and deposited in numerous mucous membranes and tissues. Granuloma formation around eggs, in particular in the liver, leads to the development of severe fibrotic and often irreversible lesions.

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Although active drugs, such as Praziquantel are available, evidence is now accumulating that, while they can reduce the overall incidence of severe forms of the disease, they do not prevent reinfection, have little effect on already developped hepatosplenic manifestations and do not significantly affect transmission.

Unlike protozoan parasites, schistosomes, as metazoans, do not replicate in their vertebrate hosts. It is agreed, on the basis of experimental and epidemiological studies, that a significant but partial reduction, estimated around 60 percent, of the worm burden following infection would considerably reduce pathology and affect parasite transmission.

Recent epidemiological studies (Butterworth et al. 1985, Hagan et al. 1991) have now clearly established that protective immunity in chronically exposed human population is slowly built up and begins to be expressed after the age of puberty. Children, who get infected, as soon as they can walk, will know before they reach adolescence a long period of susceptibility to multiple reinfections and will represent both privileged targets for the development of the disease and major actors of transmission.

It therefore appears that a vaccine strategy which could lead to the anticipated induction of effector mechanisms reducing the level of reinfection and ideally parasite fecundity would deeply affect the incidence of pathological manifestations as well as the parasite transmission potentialities.

On the basis of these general principles, our strategy has aimed, in a first phase, at the identification of effector and regulatory mechanisms of the immune response to schistosomes in experimental models and in human populations.

EFFECTOR AND REGULATORY MECHANISMS

Extensive studies performed in our laboratory have attempted to a detailed analysis of the humoral components and of the cellular partners involved in *in vitro* killing of target schistosome larvae, namely schistosomula. Using the rat as a model system in parallel with cytotoxicity assays in humans and in primates, we identified novel ADCC mechanisms involving proinflammatory cell populations (macrophages, eosinophils and platelets) as cellular partners and unusual antibody isotypes such as IgE or a subclass of IgG with anaphylactic properties, like rat IgG2a in the particular case of eosinophils (Capron et al. 1987). These observations of IgE dependent cell mediated killing, which could be confirmed in human schistosomiasis and in primates (Joseph et al. 1978, Capron et al. 1984) remained however limited at this stage to *in vitro* experiments thus raising the problem of their *in vivo* relevance. Indeed the essential question brought by these *in vitro* ADCC mechanisms was related to the implication, so far unsuspected, of anaphylactic antibody isotypes and specially IgE in the mechanisms of protection against metazoan parasites. The production of monoclonal antischistosome antibodies of the rat IgE and IgG2a isotype led to the demonstration of their high protective capacity by passive transfer (Grzych et al. 1982, Verwaerde et al. 1987). Together with the diminished protection passively conferred by IgE depleted immune rat serum and abrogation of immunity after anti m and anti e antibody treatment of neonate rats (Bazin et al. 1980, Kigoni et al. 1986), evidence was then accumulated that IgE, at least in rats, could have a more beneficial function than being mainly involved in deleterious allergic manifestations. The relevance of these experimental findings to human immunity to schistosomes have been very recently confirmed by three independent immunoepidemiological studies which have brought convergent evidence for a protective role of IgE antibody in human infection. Studying the rate of reinfection after treatment in a community exposed to *Schistosoma haematobium* infection in the Gambia, Hagan et al. (1991) have demonstrated a positive correlation between the specific IgE antibody response to worm antigens and the acquired resistance to reinfection. Multiple regression analysis show in particular that the risk of reinfection is ten times more likely when IgE antibodies are absent or in the lowest quintile. Similar findings have been made by Dunne et al. (1992) in a community exposed to *S. mansoni* infection as well as by Rihet et al. in Brazil (1991). Without excluding the possibility of the participation of additional mechanisms, as mentionned later in this review, specific IgE antibody response appears therefore as a strong correlate of protective immunity in humans, confirming the views we have expressed for many years regarding the unsuspected functions of this class of antibody (Capron & Dessaint 1985, Capron et al. 1987).

Among the various mechanisms that regulate the expression of protective immunity, one stems from isotypic regulation itself. Evidence for the selective production of defined antibody classes during the course of experimental schistosome infection in rats raised questions about the functions of other isotypes shown not to be directly implied in killing pathways. The decrease in immunity observed at certain periods of the infection in rats indeed is not related to a sharp decrease in antibody production but is concomitant with the appearance of non anaphylactic IgG subclasses. A representative IgG2c monoclonal antibody was shown to inhibit the capacity of an IgG2a mono-

clonal antibody both to induce eosinophil dependent killing of schistosomula and to confer passive protection *in vivo* (Grzych et al. 1984). The concept of blocking antibody was supported by the observation that this IgG2c monoclonal antibody can inhibit the recognition by the protective IgG2a monoclonal antibody of the carbohydrate moiety of a major surface glycoprotein of schistosomula described as gp 38 (Grzych et al. 1984).

The possibility that a similar phenomenon might be important in humans infected by *S. mansoni* was first indicated by the observation that susceptibility to reinfection after treatment of school children is significantly correlated with the presence of high levels of antibodies that inhibit the binding to the major gp38 schistosomulum surface antigen of the protective monoclonal IgG2a antibody. In addition, IgM and IgG2 antibodies isolated from the sera of various individuals directly block the eosinophil-dependent killing of schistosomula mediated by IgG antibodies from the same sera. IgM antibodies with specificity for schistosomulum surface antigens are present in higher levels in the young susceptible children than in the older, resistant subjects (Khalife et al. 1986, Butterworth et al. 1987). More recently, analysing the isotypes of antibodies to a recombinant protective protein (Sm28 GST) and its derived synthetic peptides, we found a significant correlation between susceptibility to reinfection to *S. mansoni* in humans and increased production of IgG4 antibodies to Sm28 and its defined B cell epitopes (Auriault et al. 1990). Consistently, in the framework of their studies, Hagan et al. (1991), and Dunne et al. (1992) have also shown a clear correlation between IgG4 antibody response to schistosome antigens and increased susceptibility to reinfection. More strikingly Dessein et al. (personal communication) have shown in Brazil that the association of low levels of IgE antibodies to *S. mansoni* with high IgG4 levels resulted in an increase of over 100-fold in susceptibility to reinfection. The main message from these studies is that in human schistosomiasis blocking antibodies are important components of the clinical expression of acquired resistance at its early stages, and afterwards clinical expression of immunity is positively correlated to the presence of detectable IgE antibody response to schistosomes. Such indications are obviously in the heart ot the design of defined antischistosome vaccines.

STRATEGY TOWARD VACCINE: BACKGROUND

On the basis of these concepts, we have developped during the last five years extensive investigations aiming at the identification and the molecular characterization of potentially protective antigens against schistosomiasis. The genes encoding several *S. mansoni* proteins have now been cloned in our laboratory, among which one of them initially named P28 (Balloul et al. 1987a) appears as a promising vaccine candidate. After its successful cloning in collaboration with Transgene, P28 was identified as a glutathion S.transferase (GST) (Taylor et al. 1988) and distinct in its molecular structure of a GST recently cloned from a *S. japonicum* cDNA library (Davern et al. 1987). Sm 28 GST has been expressed in various vectors, including *Escherichia coli*, *Saccharomyces cerevisiae*, and the Vaccinia virus. Vaccination experiments performed with the highly purified native protein indicated a level of protection close to 70 % in rats, 50 % in the mouse and in hamsters (Balloul et al. 1987b). Immunization performed with the recombinant protein in the presence of aluminium hydroxyde confirmed the initial results and led to a mean protection of over 50 % in rats and 40 % in mice (Balloul et al. 1987b). Several vaccination experiments were undertaken in baboons and a very significant protection up to 80 % could be obtained in some animals. However a large degree of individual variation was noticed and the mean protection observed was 42 % (Boulanger et al. 1991).

During these preliminary experiments, our attention was drawn to the existence, even in the very partially protected animals, of a significant decrease in the size and the volume of egg granulomas in the liver, whereas a mean reduction of 68 % of fecal egg output per female worm and per day was noticed. Similar observations were made in the monkey *Patas patas* immunized against *S. haematobium* infection. A dramatic decrease of urinary bladder lesions studied by ultrasound tomography during a period of eight months was observed in immunized animals compared to controls. More strikingly, eggs collected from vaccinated monkeys showed a marked decrease (85 %) in their hatching capacity and in the viability and infectivity (58 %) of the miracidia (Boulanger et al. 1991).

The use of appropriate monoclonal antibody probes to Sm 28 GST epitopes has recently allowed to relate the antifecundity effect observed after immunization to the inhibition of expression of the GST enzymatic activity of Sm 28 GST. Indeed results obtained both *in vitro* and *in vivo* indicate that a monoclonal antibody which inhibits the enzymatic activity confers, with a significant protection against challenge, a dramatic reduction in egg laying and egg viability whereas, in contrast, another protective monoclonal antibody which does not inhibit the enzymatic activity confers protection in reducing worm burden but has no effect on egg production and viability (Xu et al. 1991).

The mapping of the major epitopes of the mol-

ecule has led to the identification of the major role played by the N and C terminal domains in the expression of the enzymatic activity. The construction of corresponding synthetic peptides has allowed, after immunization, to decrease by 70 % parasite fecundity and egg viability. In this context the C. terminal epitope (190-211) appears of particular interest for the optimization of an anti parasite fecundity vaccine. In parallel, an immunodominant epitope associated with protection against challenge in experimental models and acquired resistance in human population has been identified. The immunization with an octameric construction of the corresponding peptide (115-131) has led to significant degree of protection in rats (Wolowczuk et al. 1991).

The study of the immunological mechanisms, underlying the inhibition of parasite fecundity and of egg viability has revealed the existence of an unsuspected mechanism related to the neutralising activity of IgA antibodies.

Recent studies performed in human populations have revealed a close association between the production of IgA antibodies to Sm 28 GST, their neutralising activity of the enzymatic function of the molecule and the age dependent decrease in the egg output observed in human population in parallel with acquisition of immunity (Grzych et al. 1993). Monoclonal IgA antibodies have been raised in mice immunized with rSm28 GST and their epitopic specificity has been determined. Passive transfer of IgA mAb recognizing the central domain (AA 115-131) of Sm28 GST induced up to 90 % protection. Passive transfer of IgA mAb directed against the enzymatic site of the molecule (AA 10-43 or AA 190-211) induced a reduction in the number of tissue eggs (45 %) without effect on worm burden.

It therefore appears that in terms of vaccine strategy against schistosomiasis immunization with Sm 28 GST, might achieve two complementary goals in human population: a) A partial but significant reduction of the worm population resulting from infection or reinfection; b) A significant reduction of pathological consequences by a marked decrease in parasite fecundity and egg viability, this effect affecting directly transmission potentialities of the disease.

It also appears, both from the study of experimental models and of human populations that at least two distinct immunological mechanisms, for which the cellular components remain to be defined, may account for these two effects. For the first, IgE antibodies appear as a major humoral component of acquired resistance to reinfection whereas for the other, IgA antibodies appear as a major humoral factor affecting parasite fecundity and its pathological consequences. As it could be expected immunity to such complex organisms as schistosomes is obviously multifactorial in nature and there is no a priori reason to think that successful immunization against this pathogen can be achieved though the elicitation of a single effector mechanism.

UPDATE AND PERSPECTIVES

The relevance of our observations to vaccine strategy has been recently confirmed by vaccination experiments performed in Sudan against cattle schistosomiasis due to *S. bovis*. This model was chosen because of many common features with human infection by *S. mansoni*.

In collaboration with A. Bushara and M. Taylor (Bushara et al. 1993), we could show that immunization of calves with *S. bovis* GST results in a dramatic reduction in egg production and tissue egg count (over 80 %) and acquisition of resistance to a lethal infection. These results confirmed in a natural host that the major effect of immunization with schistosome GST is to very significantly reduce parasite fecundity. They also open feasible perspectives for a veterinary vaccine against schistosomiasis in a near future and allow to consider the acceptability of Phase I trials in human populations. In more recent experiments, immunization of goats with the recombinant GST from *S. bovis* (rSb28 GST) in presence of complete Freund adjuvant resulted in a significant reduction in the worm burden (48 %). Individual levels of protection could reach up to 80 %. Unlike the controls, the immunized animals did not experience weight loss during the acute phase of infection. However, schistosome fecundity was not affected by that protocol (Boulanger et al., submitted). These results while confirming the vaccine potential of the *S. bovis* recombinant GST against veterinary schistosomiasis also clearly illustrate the striking differences in the result of immunization between distinct animal species.

At the same time, the molecular cloning and the full sequence of the chromosomal gene of Sm28 GST (McNair et al. 1993), together with the recent crystallisation of the Sm28 GST (Trottein et al. 1992) allows the study of its 3D molecular structure and new approaches toward molecular design of an optimal vaccine.

The recent cloning in our laboratory of the GST from *S. haematobium* and *S. bovis* (Trottein et al. 1992) has allowed a comparative analysis of the GST sequences of the various schistosome species. Although overall predicted aminoacid sequences identities are above 90 % between the species, crucial differences exist compared to defined Sm28 GST epitopes. Notably, a single base change determining an aminoacid substitution, leads to the non-recognition of Sh28 GST by antibodies di-

rected against the protective epitope 115-131 of the *S. mansoni* protein.

In contrast, the demonstration of the high degree of conservation of the C terminal domain among the various species of schistosomes (Trottein et al. 1992) opens the possibility that a cross-specific anti-fecundity vaccine might be achieved.

Recent experiments, in *P. patas* monkeys, appear to support this hypothesis (Boulanger et al., submitted). Indeed, whilst heterologous immunization of these monkeys with the recombinant Sm28 GST did reduce the number of worms after experimental infection with a human strain of *S. haematobium* urine and fecal egg excretions were significantly reduced during one year of observation respectively by 46 and 53 per cent.

The demonstration of the so far unsuspected function of IgA antibodies in schistosomiasis and their potential role in protective immunity has paved the way to new possibilities of immunization strategies through mucosal routes. Several approaches have now been devised.

Assessment of muramy dipeptide (MDP) to enhance specific secretory immune response has been undertaken. Administration of this immunostimulant product by oral route during systemic immunization with Sm28 GST enhanced the production of specific secretory IgA. These antibodies were shown to be effective in inhibiting enzymatic activity of the recombinant protein. In these experiments, anti-Sm28 GST secretory IgA production was correlated with the protective activity (60 %) against challenge infection in the mouse model.

Several models of synthetic vectors including liposomes have been investigated for their capacity to optimize mucosal response against the recombinant Sm28 GST. Preliminary experiments using oral administration indicated that liposome associated Sm28 GST was able to elicit a strong specific mucosal IgA response associated with a significant reduction in the worm burden (53 %) in the mouse.

One of the most appealing possibilities for the development of the new mucosal vaccines appear to rely in modified or attenuated live vectors which can be used to express genes encoding the candidate vaccine protein. A first model has been developed in collaboration with the laboratory of C. Hormaeche at Cambridge University (U.K.).

Recombinant Aro-A *Salmonella typhimurium* expressing the Sm28 GST as a fusion protein with the C fragment of tetanous toxin (Tet-C) has been used to immunize Balb/C mice by both i.v. and oral route. Both procedures gave an equally strong humoral response. A marked secretory IgA response to Sm28 GST was detected after oral administration and a significant reduction in the worm burden (48 %) was observed.

Taken altogether, these preliminary results indicate at least the feasibility of a novel and attractive approach towards oral immunization against a systemic parasitic disease.

From experimental models to human populations, from the bench to endemic areas, studies performed during the last 15 years have revealed at the level of effector mechanisms of immunity, immunoregulation and pathogenesis, novel modalities, the interest of which extend far beyond the field of schistosomiasis (Capron & Dessaint 1992 , Capron 1992). These studies seem presently to represent a promising approach towards the possible immunological control of one of the major human parasitic disease through the identification not only of potentially protective antigens but also of the components of the immune response which vaccination should aim at inducing.

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