Schistosoma mansoni Irradiated Cercarial Vaccine: Identification of Changes in Developing Parasites Which Confer Increased or Decreased Susceptibility to Vaccine Immunity

A proposal submitted for preliminary consideration to AID

April 9, 1981

by Allan H. Smith PhD

Schistosomiasis is estimated to afflict ten percent of the world population and is ranked second only to malaria as a cause of severe morbidity and mortality. Due to the nature of the parasite life cycle, it is particularly prevalent in underdeveloped countries where a cultural dependence on canals or streams exists. Thus, development of an anti-schistosomal vaccine providing long-lasting protection is essential to control of the disease, due to the likelihood of frequent infection. Present drug treatment protocols produce very unpleasant side effects and pharmacological cure of infection does not produce long-lasting immunity to reinfection.

At present, the most promising candidate for a human vaccine is the irradiated cercarial vaccine, first described by Minard et al. (Am. J. Trop. Med. Hyg. 27:76-86, 1978), where immunity is induced by infection with ^{60}Co-irradiated cercariae (the free-swimming larval stage of the parasite). First developed in the mouse model, the vaccine produces long-lasting immunity with no apparent pathological complications. The present vaccine affords 70 to 80 percent protection against challenge infection in several animal models and thus needs to be improved before human trials can be realistically considered. Utilizing the mouse model of the irradiated cercarial vaccine, I intend to determine whether the incomplete immunity afforded by the vaccine is due to the ability of a fraction of the developing parasites to rapidly acquire resistance to vaccine immunity, similar to the resistance displayed by adult worms and reflected in their acquisition of host antigen and lack of expression of schistosomal antigens. In addition, I intend to focus on the importance of specific schistosomal antigens in the acquisition of increased

resistance or susceptibility to vaccine immunity, thereby determining which antigens deserve greater consideration in attempts to increase vaccine immunity. Lastly, I will further characterize the mechanism of vaccine immunity by determining if other models of anti-schistosomal immunity evoke changes in the developing parasite population which confer increased resistance or susceptibility to the vaccine.

The proposed research employs a system whereby the parasite is introduced into a primary (1') host on Day 0 via percutaneous infection with cercariae; 6 to 10 days later, lung stage somules will be isolated from the 1' host and transferred i.v. to a secondary (2') host to complete their maturation. Those lung somule stage parasites which have survived in the 2' hosts and matured into adult worms will be collected and counted 5 to 6 weeks after transfer into the 2' host.

In each experiment there will be a normal 1' host group (1'N) and an experimental 1' host group (1'X), a normal 2' host group (2'N), and a vaccinated 2' host group (2'V). As shown in Figure I, lung somules isolated from the 1' host of each group will be pooled (1'N pool, 1'X pool) and injected into both types of 2' hosts in a crisscross pattern (Fig. I) leading to four types of 2' hosts -- $2'N_X$, $2'V_X$, $2'N_N$, $2'V_N$. The percent of survival in the 2' host will be determined by dividing the adult worm recovery of each 2'V by the adult worm recovery of the corresponding 2'N. This ratio will be called the survival index (SI) and each experiment will generate two survival indices: $2'V_X/2'N_X=SI_X$, $2'V_N/2'N_N=SI_N$ reflecting lung somules isolated from 1'X and 1'N, respectively.

Each SI is a description of the effectiveness of vaccine immunity on that particular lung somule pool. If the survival indices differ significantly such that SI_X is greater than SI_N, this will be a reflection of the fact that the initial development in 1'X somehow produced a lung somule population better able to resist the vaccine. Thus, a selective event would have taken place in 1'X which generated this vaccine-resistant population.

If SI_X is less than SI_N, it will be considered that development in 1'X somehow generated a somule population more susceptible to vaccine immunity. This would indicate that the experimental manipulation of 1'X provided an environment for the developing parasite which complemented or potentiated susceptibility to vaccine immunity.

If SI_X is not significantly different from SI_N, it will be considered that early development in 1'X had no irreversible effect on the somule population with regard to vaccine susceptibility. Such manipulation of 1'X where SI_X equals SI_N will then be regarded as having little vaccine-related effect on developing somules.

In contemplating the various aspects of this system, it must be remembered that what one is actually analyzing is a <u>population</u> of lung somules and not individual parasites. Thus, a somule population isolated from 1'X may have SI_X greater than SI_N, indicating that the population is better adapted to vaccine survival; yet the experimental manipulation of 1'X may itself be lethal for somules, killing most of them before the lung stage. The survivors of this experimental manipulation in 1'X would have been selected for some quality which promotes vaccine resistance. Therefore, having identified a

manipulation of 1'X which causes SI_X greater than SI_N, it should be possible to repeat this manipulation of 1'X to then obtain a lung stage somule population which has been selected for vaccine resistance. This population of lung somules could then be analyzed to determine what changes account for the increased vaccine resistance.

The initial study to be performed will determine whether, in vaccinated mice, passage of infecting cercariae to the lung stage confers any selective advantage on the somule population. Simply stated, these experiments will employ 1'X where the experimental manipulation is vaccination. The importance of this study is that it will determine if, during development in the vaccinated host, survival is favored for those somules that have acquired increased resistance to the deleterious effects of vaccine immunity. In the system I have described, such a selective advantage would be acquired by developing parasites by the lung somule stage.

Using the experimental description of Figure I, 1'X would be previously vaccinated mice, the same as 2'V. Thus, a result where SI_X is greater than SI_N indicates that early development in vaccinated mice does have a selective effect on the somule population, favoring the survival of those capable of resisting vaccine immunity. If it turns out that SI_X equals SI_N, then it would appear that no selective effect is evident, indicating that resistance to the vaccine is not acquired by the lung somule stage. If SI_X is less than SI_N , then it can be concluded that early passage in vaccinated mice confers no selective advantage. Furthermore, it would also seem very likely that with SI_X less than SI_N, the deleterious effects of the vaccinated host on somule

survival operates for a prolonged period, certainly past the lung somule stage, and the decrease in SI_X -- that is, the greater susceptibility to vaccine immunity -- is due to prolonged exposure to vaccine immunity (in both 1'X and 2'V). Therefore, whatever the results of this study are, they have enormous relevance to the actual mechanism of vaccine immunity since at present it is not known if vaccine immunity only acts on developing somules for a discrete period of time or is continually threatening their survival; that is, whether subversion of anti-parasite immunity during the early phase of parasite development in the vaccinated host -- before the lung stage -- is more crucial to survival of the parasite than is subversion of immunity at a later stage.

Depending upon the results of this initial study, a number of qualifying experiments are indicated. For instance, if SI_X is greater than SI_N or SI_X is less than SI_N, it will be important to determine if the initial effects of somule development in 1'X can be reversed by culture <u>in vitro</u> for 48 hours (Fig. II). This will show whether the early changes in 1'X are reversible or permanent.

The second phase of investigation employing this system deals with the effect of immunization of 1'X against various schistosome antigens. The experimental manipulation of 1'X will then be immunization with schistosomal antigen preparations, or passive transfer of serum from immunized or infected/cured animals or hybridoma antibodies directed against specific schistosomal proteins (Fig. III). In each of these experiments, if SI_X is greater than SI_N, it can be concluded that a selective advantage has been given to those somules following interaction with specific antibody. Therefore, it will be important to

determine what changes have occurred in somules isolated from 1'X with respect to expression of the corresponding antigens. If it is found that SI_X is less than SI_N, it will strongly suggest that the antigen(s) involved are particularly important in the vaccine immunity and that attempts at improving the vaccine can focus on increasing immunity to these specific antigens. Thus, by employing hybridoma antibodies specific for known schistosomal antigens, it will be possible to identify those schistosomal antigens which have important roles in vaccine immunity, based on their contributing increased susceptability or resistance to the vaccine following reaction with antibody. An alternative expression of this concept is that the system will permit one to focus on the importance of specific schistosomal antigens in the vaccinated host-parasite interaction.

Depending upon the results of this study, a number of qualifying experiments would be indicated. For instance, if SI_X is greater than SI_N, can the initial effects of passage in 1'X be reversed by culture *in vitro* for 48 hours (Fig. II)? This will determine if the early changes occurring in 1'X are reversible or somewhat permanent.

The final phase of the study will focus on the relationship between vaccine immunity and other models of anti-schistosomal immunity, such as chronic infection, BCG immunization, or immunization with *Fasceola hepatica* antigens. The impetus for this research is that it will allow a comparison to be made between the effects of maturation to the lung somule stage in a vaccinated 1' host as opposed to 1'host manipulated so as to induce these other types of immunity. These experiments will follow the protocol set up in Figure I, with 1'X being a chronically

infected mouse or a BCG or Fasceola hepatica immunized mouse. As previously explained, SI_X and SI_N values will be determined for each experimental manipulation and compared to SI_X, SI_N values obtained from the first phase of this project where 1'X was a vaccinated mouse.

The interpretation of the results obtained in this final phase will depend largely upon the results obtained in the first phase. If for a given manipulation of 1'X, such as chronic infection, for example, the relationship between SI_X and SI_N is the same as that achieved for vaccinated 1'X in the first phase of this project, than it can be assumed that while developing to the lung stage, somules are subjected to the same type of selective pressure by chronic immunity as by vaccine immunity. This would lead to the assumption that both types of immunity effect similar changes in the developing somule population. Lung somules from both chronically infected and vaccinated 1'X would then be examined to determine what these similarities are, leading to a better understanding of vaccine immunity. If, however, the relationship between SI_X and SI_N for chronically infected 1'X is the exact opposite from that observed for vaccinated 1'X, then it will be important to determine how the development to the lung somule stage differs between chronically infected and vaccinated 1'X. This difference would describe features of increased or decreased susceptibility to vaccine immunity. This third phase of the study is then an attempt to further elucidate the mechanism of vaccine immunity.

The experimental transfer system described in this proposal will therefore be employed to study mechanisms by which developing somules acquire increased or decreased susceptibility to vaccine immunity, to

focus on the role of specific parasite antigens in host-parasite interactions which relate to vaccine immunity, and also to detect similarities and differences between other models of anti-schistosomal immunity and the irradiated cercarial vaccine.

The intent of the proposal is to identify maturational changes in the somules which confer resistance to vaccine immunity and thus orient further attempts in vaccine improvement toward the delay of these changes.

The research in this proposal will be conducted at the Biomedical Research Institute (BRI) of the American Foundation for Biological Research in Rockville, Maryland. The life cycle of Schistosoma mansoni has been successfully maintained at BRI for nine years and this is one of the few institutions capable of supplying the large number of cercariae necessary for these experiments. The laboratories at BRI, originally designed for research in schistosomiasis, provide ample space and require no renovation and minimal equipment. In addition, the staff of BRI includes Drs. Margaret Stirewalt and Fred Lewis, both distinguished researchers in schistosomiasis who will be available for consultation and collaboration. A projected timetable for this work is given in Table I.

Figure I

Diagram of Basic Experimental System

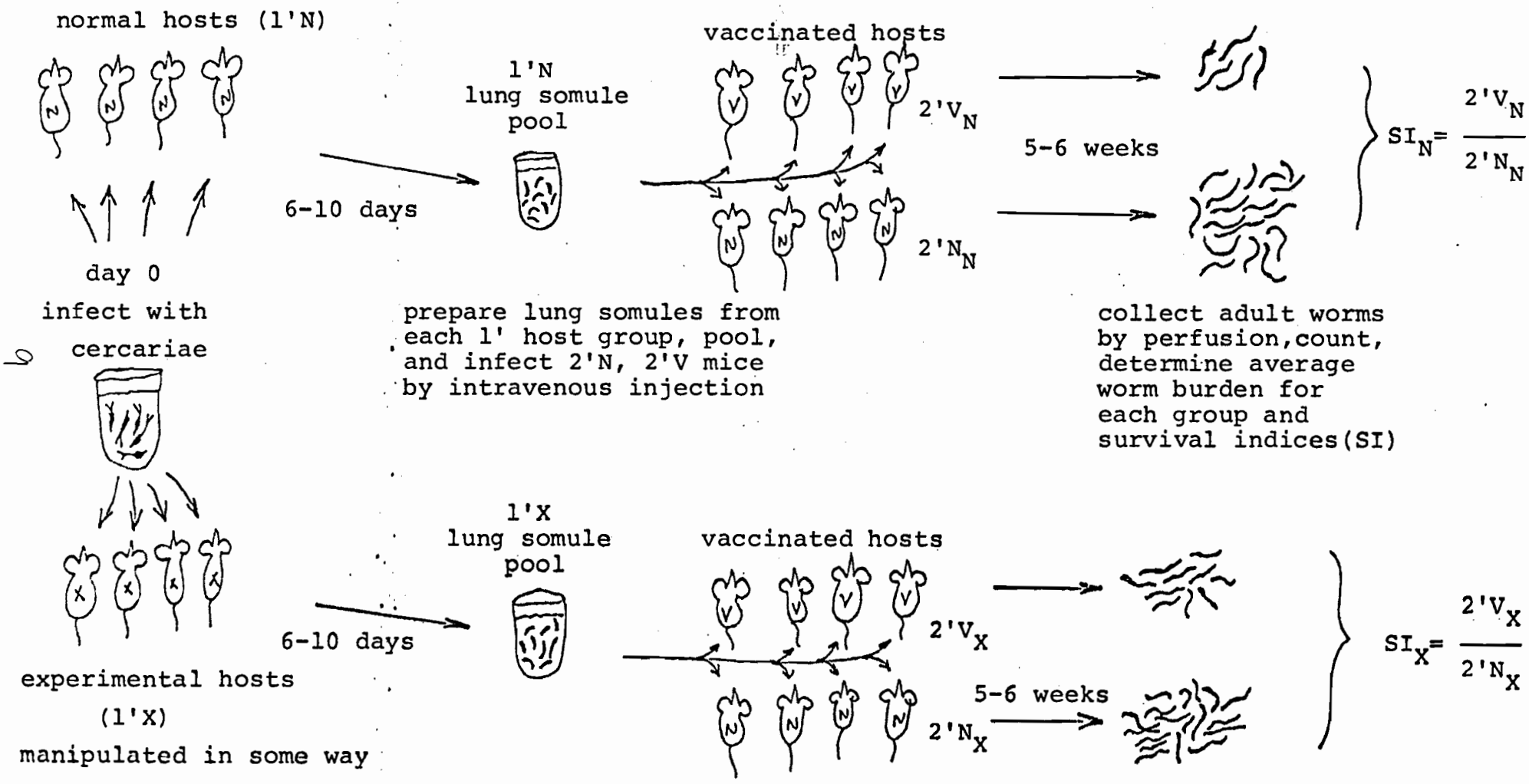

Figure II

Protocol to Attempt to Reverse Effects of Development

in 1'X by *in vitro* Culture

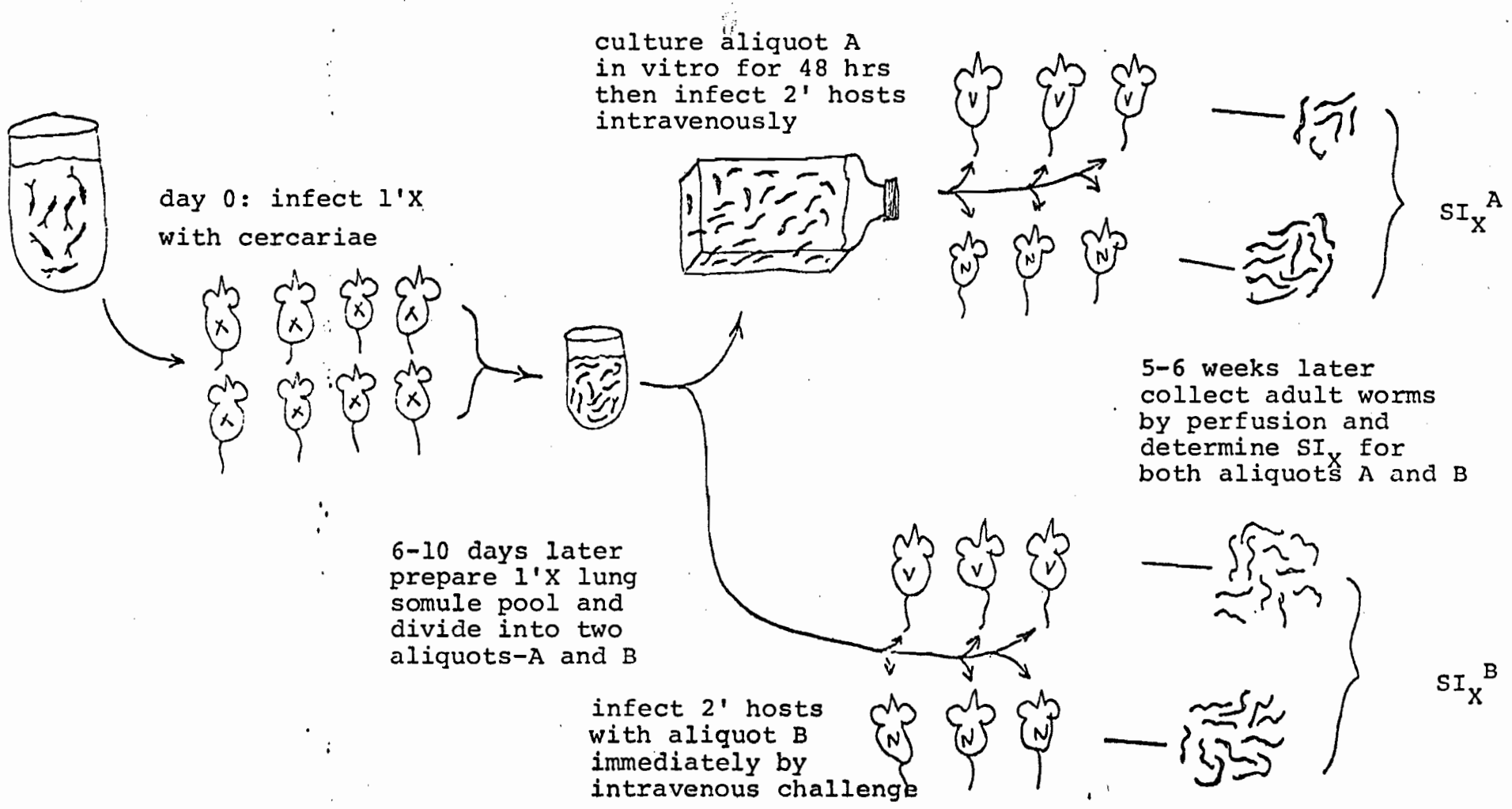

Figure III

Protocol for Preparing 1'X by Immunization with Worm Antigens or by Passive Transfer of Immune Serum

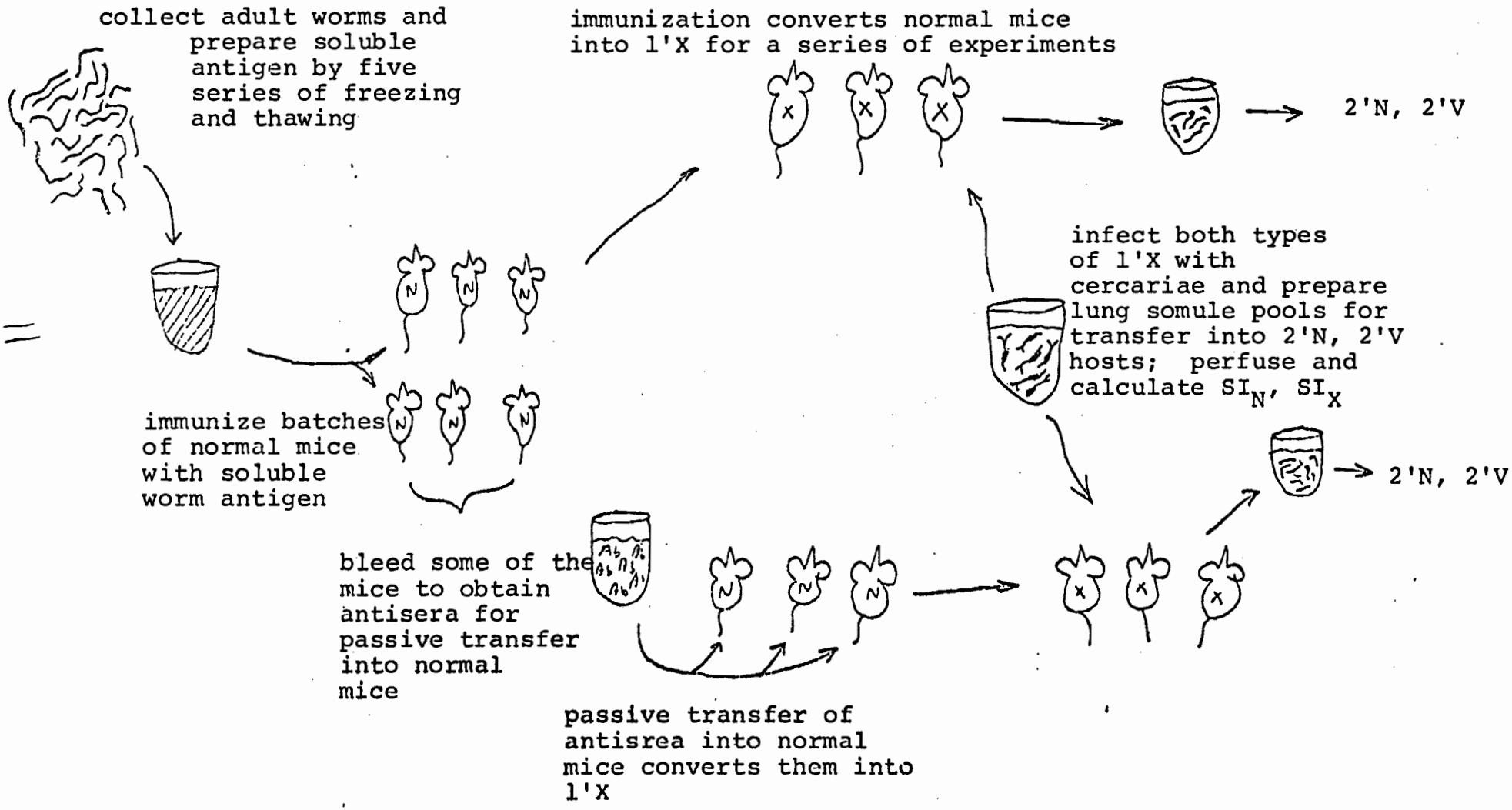

Table I

Projected Timetable for Research

Project	Duration (months)											
	3	6	9	12	15	18	21	24	27	30	33	36
Determine kinetics of lung somule recovery in normal and vaccinated hosts. . . .	XXX											
Vaccinate mice with irradiated cercariae	XXXXXXXXXXXXXXXXXXXXXXXXXXXXXX											
Collect adult worms and prepare soluble antigen	XXXXXXXXXXXXXXXXXXX											
First phase of research: 1'X is vaccinated mouse			XXXXXXXXXXXXXXXX									
In vitro culture of 1'X lung somules from first phase . .					XXXXXXXXXXX							
Immunize mice with soluble antigens, prepare monoclonal antibody by cell fusion(hybridomas), collect antisera . .			XXXXXXXXXXXXXXXXXXXXXX									
Second phase of research: 1'X immunized with soluble antigen or recieves immune serum or monoclonal antibody					XXXXXXXXXXXXXXXXXXXXXXXXXX							
Set up alternate models of immunity such as chronic infection or BCG injection . . .					XXXXXX							
Third phase of research: use alternate models of immunity as 1'X						XXXXXXXXXXXXXXXXXXXX						

ALLAN H. SMITH - LETTER OF INTENT

Schistosomiasis is estimated to afflict ten percent of the world population and is ranked second only to malaria as a cause of severe morbidity and mortality. Due to the nature of the parasite life cycle, it is particularly prevalent in underdeveloped countries where a cultural dependence on canals or streams exists. Thus development of an anti-schistosomal vaccine providing long-lasting protection is essential to control of the disease, due to the liklihood of frequent infection. Present drug treatment protocols produce very unpleasant side effects and pharmacological cure of infection does not produce long-lasting immunity to reinfection.

At present, the most promising candidate for a human vaccine is the irradiated cercarial vaccine, first described by Minard et al. (Am. J. Trop. Med. Hyg. 27: 76-86, 1978), where immunity is induced by infection with ^{60}Co-irradiated cercariae (the free swimming larval stage of the parasite). First developed in the mouse model, the vaccine produces long-lasting immunity with no apparent pathological complications. The present vaccine affords 70-80% protection against challenge infection in several animal models and thus needs to be improved before human trials can be realistically considered. The proposal I intend to submit for A.I.D. sponsorship outlines a research protocol designed to identify those aspects of parasite development and the host-parasite interaction which are the causes of the less than total effectiveness of the irradiated cercarial vaccine. This research is crucial to further vaccine development since it will identify the mechanisms by which the parasite evades vaccine immunity. Furthermore, features of the host-parasite interaction which stimulate these mechanisms will be investigated.

Previous research in the murine model of schistosomiasis has implied that during parasite maturation tegumental changes in the developing worm (somule) renders it resistant to vaccine immunity. The consistent survival of 20-30% of a challenge infection in vaccinated animals is therefore likely to depend on the ability of this fraction of the somule population to develop these changes rapidly enough to become resistant to the vaccine. Thus surviving ~~20-30%~~ immature parasite stages isolated from vaccinated mice should represent a vaccine resistant population when compared to the same parasite stage isolated from non-immune hosts. When injected back into vaccinated hosts, somules originally isolated from vaccinated mice should survive better than somules isolated from normal mice. The initial focus of the proposal will be to determine whether or not this occurs. Somules isolated from normal and vaccinated mice (1' hosts) will be transferred into a second group of normal and vaccinated mice (2' hosts) and the survival of both somule populations in the 2' hosts will be compared. These experiments will reveal whether a stage of parasite maturation confers vaccine resistance - a very important consideration for further vaccine improvement.

The 1' → 2' host transfer system will also be employed to determine if other manipulations of 1' hosts, such as passive transfer of immune serum or immunization with worm extracts can produce a vaccine resistant somule population. Those manipulations which ~~do will identify~~ reflect aspects of the host-parasite interaction which stimulate worm survival. induce vaccine resistance Attempts at vaccine improvement can then be directed at reversing those aspects of the host-parasite interaction which promote worm survival.

The intent of the proposal is to identify maturational changes in the somules which confer resistance to vaccine immunity, to investigate aspects of the host-parasite interaction which promote these changes and ~~this~~ thus orient further attempts in vaccine improvement toward the delay of these changes.

The research in this proposal will be conducted at the Biomedical Research Institute (BRI) of the American Foundation for Biological Research in Rockville, Md. The life cycle of Schistosoma mansoni has been successfully maintained at BRI for nine years and this is one of the few institutions capable of supplying the large number of cercariae necessary for these experiments. The laboratories at BRI, originally designed for research in schistosomiasis, provide ample space and require no renovation and minimal equipment. In addition, the staff of BRI includes Drs. Margaret Stirewalt and Fred Lewis, both distinguished researchers in schistosomiasis who will be available for consultation and collaboration. The estimated budget is also based on a two-year tenure and is presented in Table II. The costs are figured at the current BRI overhead rate of 119.53% of salary and realistically reflect the cost of sponsorship of this research.